INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505
Available Online at www.journalijcar.org
Volume 14; Issue 12; December 2025; Page No.583-586
DOI: http://dx.doi.org/10.24327/ijcar.2025.0586.0093

Subject Area : Nizamabad

COMPARATIVE EVALUATION OF THE EFFECT OF VARIOUS CHELATING AGENTS ON THE MICROHARDNESS OF RADICULAR DENTIN: AN IN VITRO STUDY

Dr. Pola Samba Shiva Rao, Dr. Janavathi, Dr. Jarupula Deepa, Dr. Sowmya Kotha, Dr. Rohith Shinde, and Dr. K. Sai Supraja Reddy

Meghna Institute of Dental Sciences, Nizamabad

ARTICLE INFO

Received 15th November, 2025
Received in revised form 29th November, 2025
Accepted 17th Dcember, 2025
Published online 28th Dcember, 2025

Key words:

Chelating agents,EDTA,HEBP,Microhardness, Sodium gluconate

ABSTRACT

Aim: The Aim of this study is to evaluate and compare the effect of 17% liquid EDTA, 16% Sodium Gluconate and 9% HEBP on the microhardness of root dentin. **Materials and Methods:**Thirty mandibular premolars were selected and randomly assigned to one of the three groups(n = 10). Decoronate the teeth up to CEJ, specimens were prepared up to F3 (ProTaper Universal). Specimens were sectioned longitudinally to expose root dentin surface. The test group was one half, and the control group was the other half. Group 1: 17% liquid EDTA, Group 2: 16% Sodium gluconate, and Group 3: 9% HEBP. Measurement of microhardness was done using Vickers indenter with a load of 200 g and a holding time of 10 seconds. Statistical analysis: The data obtained was Statistical analyzed by using one-way ANOVA and post-hoc Tukey test at $P \leq 0.05$. **Results:** The mean difference in microhardness of EDTA is more followed by sodium gluconate and HEBP. **Conclusion:**Using all three chelating agents resulted in a noticeable decrease in microhardness. HEBP, on the other hand, reduced microhardness the least and may be a useful chelating agent in endodontics.

INTRODUCTION

Instrumentation of the root canal by using rotary or hand files finally leads to the formation of a smear layer which consists of organic and inorganic debris along with the bacteria and their byproducts which are already present in the infected root canal space.1Mechanical instrumentation of the root canal system generates a smear layer of 1–5 µm thickness on the canal walls.2A combination of instrumentation along with the use of irrigants has been suggested to remove the smear layer. The protocol for smear layer removal is by continuous rinsing using 0.5%–5.25% sodium hypochlorite (NaOCl) followed by 17%ethylenediaminetetraacetic acid (EDTA).3A single endodontic irrigant do not have all of the ideal properties of an ideal irrigant.Therefore, a combination of irrigating solutions are often used sequentially for adequate removal of the smear layer.4

The smear layer blocks and prevents the complete penetration of the intracanal medicaments into the dentinal tubules. Moreover, it prevents the adherence of the obturating materials into the dentinal tubules.5 The use of other irrigants such as sodium hypochlorite along with chelating agents such as ethylenediaminetetraacetic acid (EDTA), citric acid and maleic acid are routinely used to eradicate the smear layer. However, these irrigants can cause alterations in the chemical structure of human radicular dentin and change the calcium: phosphorous ratio of dentin of the root canal, thus affecting its microhardness.6

EDTA is an effective and strong chelating agent and its efficiency mainly depends onfactors such as concentration,type of solution, root canal length, duration of applicationand hardness of root dentin.7It demineralizes the inorganic components of the root dentin and the smear layer, which results in exposure of the collagen.3It causes dentinal erosion when used in combination with sodium hypochlorite thereby decreasing the dentin microhardness.8

A derivative of gluconic acid obtained from Zea mays (Corn) is sodium gluconate which is also used as a chelator.9It has a wide range of applications ranging from cosmetics to pharmaceuticals due to its chelating ability on calcium and other divalent & trivalent metal ions.10Its chelating ability at an alkaline pH is comparable to that of EDTA.11

A soft chelating irrigation protocol with bisphosphonates

*Corresponding author: **Dr. K. Sai Supraja Reddy**

Meghna Institute of Dental Sciences, Nizamabad

such as 1hydroxy ethylidene1, 1bisphosphonate (HEBP) also known as etidronic acid or etidronate, has been introduced as a potential alternative to EDTA or citric acid because of its chelating ability. Also, unlike other chelating agents, it does not have shortterm reactivity with sodium hypochlorite.12

The Aim of this study is to evaluate and compare the effect of 17% liquid EDTA, 16% Sodium Gluconate and 9% HEBP on the microhardness of radicular dentin.

MATERIALS AND METHODS

Thirty single rooted human mandibular premolars with single root canals which were freshly extracted and devoid of any caries, fracture, or previous restorations/filling were drawn and assigned to one of the three groups:

Group 1: 17% liquid EDTA (DENT WASH, PRIME DENTAL PRODUCTS, India)

Group 2:16% Sodium gluconate (BRM SOLUTIONS, India)

Group 3: 9% HEBP (TWIN KLEEN, MAARC DENTAL, SHIVA PRODUCTS, India).

Biomechanical preparation of root canal

Using a slow-speed diamond disc and abundant water irrigation, the chosen specimen was decorated at the cementoenamel junction. To determine the working length, a size 10 K file (Mani, Japan) was used to access the root canal of each specimen. Following each instrumentation, the canals were irrigated with 3% sodium hypochlorite (Prime Dental Products, India) and prepared up to file F3 (ProTaper Universal, Dentsply Maillefer, Switzerland). To get rid of any remaining sodium hypochlorite and debris from the root canal, a final flush with deionized water was performed.

Preparation of the specimens

After placing grooves along the long axis of the roots using a diamond disc mounted on a slowspeed handpiece under copious water irrigation, the roots were then bisected in two halves longitudinally by chisel and mallet. One half, which served as a control for the other half, was treated with the test solution.

Microhardness evaluation before exposure

Prior to each sample being exposed to the test solutions, its microhardness was assessed. A Vickers tester was used to measure the microhardness of dentin surface around 0.5 mm from the root canal space using an indenter that was loaded with 200 g and held for 10 seconds. This indentation is then utilized to determine the Vickers hardness number.

Preparation of test solutions

In this study, 17% liquid EDTA (Dent Wash, Prime Dental Products, India), 16% Sodium gluconate which was prepared at a concentration of 16% by dissolving 16 g of sodium gluconate powder in 100 ml of sterile water stabilized to a pH of 9 with 1 ml 0.1 N NaOH and a solution of 9% of HEBP (Twin Kleen, Maarc dental, Shiva Products, India) was freshly prepared to use according to manufacturer's instructions.

Microhardness evaluation after exposure

The specimens were immersed in the appropriate test solution for five minutes. To get rid of any remaining test solution, the specimens were given a final washing with deionized water. Following exposure to the test liquids, each sample's microhardness was assessed. The difference between the baseline and post-treatment values was used to compute the change in microhardness.

Statistical Analysis

The resultant data obtained were Statistical analyzed by using one-way ANOVA and post-hoc Tukey test. The level of significance was set at $P \leq 0.05$.

RESULTS

The results were tabulated.

Table 1 shows difference between the groups and also within the group.

Table 1. ANOVA TEST (Group A vs Group B vs Group C)

	Sum of Squares	df	Mean Square	F	P Value
Between Groups	115.267	2	57.633	3.973	0.031*
Within Groups	391.700	27	14.507		
Total	506.967	29			

Table 2 shows Pairwise comparison of mean difference in microhardness.

Table 2. POST HOC TUKEY's HSD TEST: Pairwise comparison of mean difference in microhardness

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
EDTA	Sodium Gluconate	0.800	1.703	0.886	-3.42	5.02
HEBP	EDTA	4.500*	1.703	0.035*	.28	8.72
Sodium Gluconate	HEBP	3.700	1.703	0.094	-.52	7.92

- The mean difference in microhardness between test and corresponding control groups was statistically significant in HEBP group vs EDTA group (Group C < Group A) (p=0.035).
- The least mean difference in microhardness between test and corresponding control groups was found in the HEBP group, followed by sodium gluconate group and EDTA group (Group C < Group B ≈ Group A).
- No statistically significant difference was found in the mean difference of microhardness between test and control groups in the EDTA group vs Sodium

Gluconate group (Group A ≈ Group B) (p=0.886); and HEBP vs Sodium Gluconate group (Group B ≈ Group C) (p=0.094).

Fig.1. Armamentarium

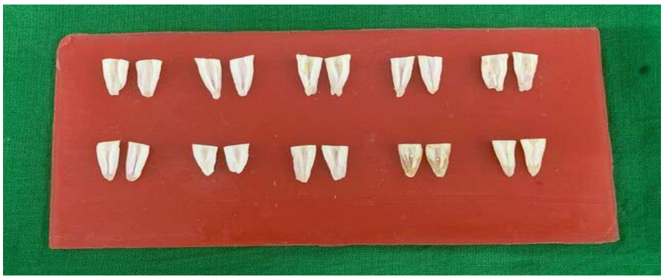

Fig.2. Longitudinal sections for each group

DISCUSSION

According to the modern methods of chemomechanical preparation, it is necessary to use chemicals on the prepared or instrumented root canal surfaces for complete removal of the smear layer. In this study, all the chelating agents showed some reduction in microhardness.

A bisphosphonate with calcium chelating qualities, HEBP shares structural similarities with the natural pyrophosphate molecule and has two phosphonate groups. These two phosphonate groups are attached to a central carbon whereas the pyrophosphate molecule has central oxygen. It is this three dimensional pyrophosphate structure which is responsible for the chelating property with divalentcations such as calcium. In our study, HEBP shows less reduction in microhardness when compared to control group. This can be ascribed due to the large amount of intertubular dentin area which becomes available for hybridization when a soft chelator is used.12Furthermore, of all the test solutions utilized, it can account for the least amount of microhardness loss because it partially depletes the surface calcium. As this HEBP being a slow chelator, the time required for its action is about 300s.13

Sodium gluconate is a polyhydroxycarboxylic acid, and its chelating ability depends on the pH of the surrounding. Its chelating property increases as the pH of the surrounding increases.Its calcium-chelating ability is through the carboxylic oxygen atom and the α-hydroxylic ligand.14It can chelate trivalent ions such as iron strongly in neutral conditions but bivalent metallic ions like calcium require a strongly alkaline pH. The high alkaline pH is necessary to release protons from the hydroxyl groups, thereby creating anionic centres in the molecule which are known to bind metals strongly.

Sodium gluconate was utilized in this investigation at an ideal pH of 9, where it produces a calcium gluconate complex only through the carboxylic oxygen atom since the pH is too low to allow the formation of anionic centres. This phenomenon has led to the selected chelating ability of sodium gluconate to chelate divalent ion such as calcium. Additionally, the decalcification effect of these chelating agents on dentin was assessed indirectly using microhardness testing, which showed that sodium gluconate surpassed EDTA due to a much smaller drop in dentine microhardness.15

EDTA has been regarded as the most widely used chelating agent for many years for removal of the inorganic debris from the root canal. Because of its chelating ability, EDTA exhibits a greater decrease in dentin microhardness in this study than other substances. Although several theories have been proposed to explain this phenomenon, one of the most accepted theories is crystalline field theory, according to this, there exists a purely electrostatic force of attraction between the central metal and the ligand. The force of attraction exerted by the central metal ions is greater than the repulsive force from the atoms of the EDTA molecule. Carboxyl groups which are present in the EDTA molecule are ionized and release hydrogen atoms and these anionic sites further compete with calcium ions thus forming a stable complex with calcium ions in dentin.16 Furthermore, EDTA softens the calcified structures present in dentin and is the major adverse effect of it, which results in reduction in microhardness of radicular dentin. Additionally, applying EDTA to dentin for an extended length of time (>1 minute) might cause negative consequences such as dentinal erosion and the breakdown of intertubular and peritubular dentin.17

Since there is currently no agreement in the literature about the use of these chelating agents, the time for all test solutions used in this study was fixed at five minutes.

One of the most basic nondestructive mechanical characterisation techniques is the measuring of a material's hardness. Dentin's microhardness can be measured using two different techniques (Knoop and Vickers). In tooth hardness research, it has been suggested that the Vickers indenter is more beneficial than the Knoop.18

The study's potential drawback may be that, because it was conducted in vitro, a smaller volume of irrigant was used during the root canal therapy process than when the specimen was submerged. Nonetheless, similar outcomes between the other irrigants were made possible by the application of uniform conditions.

CONCLUSION

HEBP proved to be a better chelating agent as compared to EDTA and sodium gluconate as it caused a low reduction in the microhardness of dentin apart from its other benefits. This effect on the microhardness of the dentin might also translate into a higher fracture resistance of the teeth treated with HEBP.

References

1. Violich DR, Chandler NP. The smear layer in endodon-

tics–a review. International endodontic journal. 2010 Jan;43(1):2-15.

2. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2002 Dec 1;94(6):658-66.
3. Zaparolli D, Saquy PC, Cruz-Filho AM. Effect of sodium hypochlorite and EDTA irrigation, individually and in alternation, on dentin microhardness at the furcation area of mandibular molars. Brazilian dental journal. 2012;23:654-8.
4. Jalan P, Purayil TP, Ballal NV, Kumar SS. Comparative evaluation of various chelating agents on the microhardness of root canal dentin: An: in vitro: study. Saudi Endodontic Journal. 2021 Jan 1;11(1):19-23.
5. Farhad AR, Barekatain B, Koushki AR. The effect of three different root canal irrigant protocols for removing smear layer on the apical microleakage of AH26 sealer. Iranian endodontic journal. 2008;3(3):62.
6. Hennequin M, Pajot J, Avignant D. Effects of different pH values of citric acid solutions on the calcium and phosphorus contents of human root dentin. Journal of Endodontics. 1994 Nov 1;20(11):551-4.
7. Poudyal S, Wei-Hong P. Effect of ethylenediamine-tetraacetic Acid (EDTA) gel on removing smear layer of root canal in vitro. Chinese Medical Sciences Journal= Chung-kuoi Hsueh k'o Hsueh tsa Chih. 2012 Sep 1;27(3):190-1.
8. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. International Endodontic Journal. 2002 Nov 1;35(11).
9. Canete-Rodriguez AM, Santos-Duenas IM, Jimenez-Hornero JE, Ehrenreich A, Liebl W, Garcia-Garcia I. Gluconic acid: Properties, production methods and applications—An excellent opportunity for agro-industrial by-products and waste bio-valorization. Process biochemistry. 2016 Dec 1;51(12):1891-903.
10. Abdelazim MH, Abdelazim AH. Effect of sodium gluconate on decreasing elevated nasal calcium and improving olfactory function post COVID-19 infection. American journal of rhinology & allergy. 2022 Nov;36(6):841-8.
11. Rabie AI, El-Din HA. Sodium gluconate as a new environmentally friendly iron controlling agent for HP/HT acidizing treatments. InSPE Middle East Oil and Gas Show and Conference 2015 Mar 8 (pp. SPE-172640). SPE.
12. Dineshkumar MK, Vinothkumar TS, Arathi G, Shanthisree P, Kandaswamy D. Effect of ethylene diamine tetra-acetic acid, MTADTM, and HEBP as a final rinse on the microhardness of root dentin. Journal of Conservative Dentistry and Endodontics. 2012 Apr 1;15(2):170-3.
13. Bedir SS, Mossa H, Hassan AM. Etidronate as a weak chelating agent on root canal dentin: An update review. J Clin Diagn Res. 2017 Dec 1;11:5-9.
14. Phadungath C, Metzger LE. Effect of sodium gluconate on the solubility of calcium lactate. Journal of dairy science. 2011 Oct 1;94(10):4843-9.
15. Karthikeyan HR, Rajakumaran A, Rajendran MR, Balaji L. Evaluation of Effect of Natural Extract Sodium Gluconate on Smear Layer and Dentine Decalcification Compared with EDTA–An In-vitro Study. European Endodontic Journal. 2023 Aug;8(4):274-9.
16. Napte BD, Srinidhi SR. Evaluation of the action of chelating agents on the microhardness ofsuperficial layer of root canal lumen dentin. World J Dent 2014;5:1248.
17. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. Journal of endodontics. 2002 Jan 1;28(1):17-9.
18. GutiérrezSalazar MD, ReyesGasga J. Microhardness and chemical composition of human tooth. Mater Res 2003;6:367

How to cite this article:

Pola Samba Shiva Rao., et al. (2025). Comparative evaluation of the effect of various chelating agents on the microhardness of radicular dentin: an in vitro study , International Journal of Current Advanced Research, 14(12), pp.583-586

****