EUROPEAN ENDODONTIC JOURNAL

ORIGINAL ARTICLE

Evaluation of Effect of Natural Extract Sodium Gluconate on Smear Layer and Dentine Decalcification Compared with EDTA – An *In-vitro* Study

Hari Raghavendar KARTHIKEYAN, Arasappan RAJAKUMARAN, Mathan Rajan RAJENDRAN, Lakshmi BALAJI

Department of Conservative Dentistry and Endodontics, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Chennai, India

ABSTRACT

Objective: Mechanical instrumentation of the root canal system generates a smear layer on the canal walls which are removed most commonly with the help of chelators such as ethylenediaminetetraacetic acid (EDTA) but can potentially cause severe dentinal erosion. Considerable research has been conducted to find an alternative to EDTA which removes the smear layer without causing dentinal erosion. The current study aimed at evaluating the ability of sodium gluconate compared with that of 17% EDTA in smear layer removal along with its effect on dentine decalcification when used as a final irrigant.

Methods: Twenty single-rooted mandibular premolars were collected and prepared based on the pre-set criteria. Following preparation, the specimens were exposed to the test solutions as a final irrigant. Then the specimens were subjected to (Scanning electron microscope) SEM analysis at 1000x for evaluating the smear layer and 5000x for evaluating the dentinal erosion, and a Vickers microhardness tester was used for evaluating the reduction in dentine microhardness post-treatment. The values obtained were analysed using SPSS software for a statistically significant difference with Mann-Whitney U test for evaluating of smear layer removal and dentinal erosion and using one-way (Analysis of variance) ANOVA test for microhardness evaluation.

Results: The smear layer removal capability of sodium gluconate was as effective as EDTA on the contrary sodium gluconate did not cause any dentinal erosion compared to EDTA with a statistically significant difference (p=0.002 in middle third and p=0.001 in apical third of the canal). Microhardness reduction caused by sodium gluconate was less compared to EDTA, however, no statistically significant difference (p=0.113) was noted.

Conclusion: Sodium gluconate, therefore, can produce a balance between smear layer removal and dentinal decalcification and can be considered a potential alternative to EDTA.

Keywords: EDTA, root canal irrigant, scanning electron microscope, smear layer, sodium gluconate

Please cite this article as:
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. Eur Endod J 2023; 8: 274-9

Address for correspondence:
Arasappan Rajakumaran
Department of Conservative Dentistry and Endodontics, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Chennai, India
E-mail:
arasappan@sriramachandra.edu.in

Received February 08, 2023,
Revised April 05, 2023,
Accepted April 26, 2023

Published online: August 03, 2023
DOI 10.14744/eej.2023.93063

HIGHLIGHTS

- Sodium gluconate is a potent calcium chelator whose smear layer removal capability is comparable to that of EDTA.
- The dentinal erosion produced by it significantly lesser than that of EDTA.
- It can produce a balance between smear layer removal and dentinal decalcification.

INTRODUCTION

The ultimate aim of root canal treatment is to prevent and treat apical periodontitis (1) and every step in the treatment is focused on eliminating the microbial load of the root canal system (2), achieved by the combination of mechanical instrumentation along with an irrigant (3). Mechanical instrumentation of the root canal system generates a smear layer of 1–5 µm thickness on the canal walls (4). The protocol for smear layer removal is a sequential rinse using 0.5%–6.15%sodium hypochlorite (NaOCl) followed by 17% ethylenediaminetetraacetic acid (EDTA) (5).

EDTA is an effective chelating agent, and its efficiency depends on several factors such as concentration, duration of application, type of solution, root canal length, and hardness of root dentine (6). EDTA indistinguishably demineralizes the inorganic constituents of the smear layer and the root dentine, with consequent exposure of the collagen (5). It produces dentinal erosion when used in conjunction with sodium hypochlorite (7) thereby decreasing the dentine microhardness.

Considerable research has been conducted to find an alternative to EDTA which provides optimum smear layer removal without dentinal erosion.(5) A derivative of gluconic acid obtained from Zea mays (Corn) is sodium gluconate which is one such chelator (8). It has a wide range of applications ranging from cosmetics to pharmaceuticals due to its chelating ability on calcium and other divalent & trivalent metal ions (9). Its chelating ability at an alkaline pH is comparable to that of EDTA (10). However, its usage has not been explored yet in the field of endodontics.

The current study aimed to evaluate the ability of sodium gluconate compared with that of 17% EDTA in smear layer removal along with its effect on dentine decalcification when used as a final irrigant.

The null hypothesis tested was that there would be no statistically significant difference between sodium gluconate and 17% EDTA in terms of (a) smear layer removal, (b) dentinal erosion and (c) microhardness reduction.

MATERIALS AND METHODS

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai - CSP/22/MAY/110/329 (approved on 19/07/2022).

Preparation of Sodium Gluconate Solution

Sodium gluconate solution was prepared at a concentration of 16% by dissolving 16 g of sodium gluconate powder (Loba Chemie Pvt. Ltd., Mumbai, India) in 100 ml of sterile water stabilized to a pH of 9 with 1 ml 0.1 N NaOH.

Evaluation of Smear Layer Removal And Dentinal Erosion

Sample size calculation

The sample size of 10 per group was determined using G*power software version 3.1.9.2 with a power of 80%. Statistical significance was set at 0.05 (p=0.05).

Teeth selection

Twenty single, straight-rooted, single-canal human mandibular premolars with fully formed roots extracted due to periodontal conditions and orthodontic requirements were included in this study. The collected teeth were stored and disinfected according to Occupation and Safety Health Administration (OSHA) guidelines. Teeth with abnormal root canal anatomy, severely curved roots, caries and previously endodontically treated teeth were excluded from the study after visual and radiographic examination by an examiner.

Sample preparation

The teeth were decoronated and standardised to a length of 15 mm using a diamond disc in a slow-speed micromotor handpiece (NSK Ltd, Tokyo, Japan). The canals were negotiated with #8, #10, and #15 K files (MANI Inc, Tochigi, Japan) and canal preparation was done using Protaper gold (Dentsply International, Inc, North Carolina, USA) rotary file till master apical size F3. Copious irrigation was done with 3% sodium hypochlorite using a side-vented needle keeping the needle 1 mm short of the apex.

Randomisation

Following canal preparation, the specimens were then randomly divided into two groups, no control group was included in the study.

- Group A – 5 ml of 17% EDTA (Desmear, Anabond Stedman Pharma Research (P) Ltd., Chennai, India) (n=10) used as a final irrigant only for 5 min (1ml/min)
- Group B – 5 ml of 16% Sodium gluconate (n=10) used as a final irrigant only for 5 min (1ml/min).

Teeth sectioning

The teeth samples were then sectioned longitudinally by placing two longitudinal grooves on the buccal and lingual aspect with a diamond disc and split into two halves with a chisel and mallet. One-half of each sample with an adequate canal portion was selected and subjected to scanning electron microscope (SEM) analysis.

Scanning electron microscope analysis

The teeth samples were then mounted on metallic stubs, which were then sputter coated with gold (JEC-3000FC, JEOL Ltd, Tokyo, Japan) enabling the surface to be electrically conductive for the SEM analysis (JEOL Ltd, Tokyo, Japan). The SEM images of the specimens were taken at 1000x and 5000x magnification steps at the coronal third, middle third and apical third levels. In total, six images were obtained for each sample. The images were analysed by a blinded investigator for smear layer and dentinal erosion based on Rödig et al. (11) criteria and Torabinajed criteria (12) respectively by a blinded examiner. The median, first quartile (Q1) and third quartile (Q3) scores of all the specimens in the coronal, the middle, and the apical third were calculated and analysed statistically using SPSS software version 22.0 (IBM, USA) with Mann-Whitney U test.

Rödig et al. (11) criteria for evaluating smear layer removal:

1. No smear layer, dentinal tubules open.
2. Small amount of smear layer, some dentinal tubules open.
3. Homogenous smear layer covering the root canal wall, only a few dentinal tubules open.
4. Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.
5. Heavy inhomogeneous smear layer covering the complete root canal wall.

TABLE 1. Median, first quartile (Q1) and third quartile (Q3) of the smear layer values of the groups

Specimen	Apical third		Middle third		Coronal third	
	Median	Q1-Q3	Median	Q1-Q3	Median	Q1-Q3
Group A	2	1–3	1	1–2	1	1–2
Group B	2	2–2	1	1–2	1	1–1
p	0.912		0.912		0.430	

TABLE 2. Median, first quartile (Q1) and third quartile (Q3) of the dentinal erosion of the groups

Specimen	Apical third		Middle third		Coronal third	
	Median	Q1-Q3	Median	Q1-Q3	Median	Q1-Q3
Group A	1	1–2	2	2–2	2	2–2
Group B	1	1–1	1	1–1	1	1–1
p	0.142		**0.002**		**0.001**	

Torabinajed et al. (12) criteria for evaluating dentinal erosion:

1. No erosion–All tubules looked normal in appearance and size.
2. Moderate erosion – The periradicular dentine was eroded.
3. Severe erosion – The intertubular dentine was destroyed and tubules were connected with each other.

Evaluation of Dentine Microhardness

Sample size calculation

The sample size of n=6 per group was determined using G*power software version 3.1.9.2 with a power of 80%. Statistical significance was set at 0.05 (p=0.05).

Teeth selection

Six single, straight-rooted human mandibular premolars with fully formed roots extracted due to periodontal conditions and orthodontic requirements were included in this study. Teeth with abnormal root canal anatomy, severely curved roots, caries and previously endodontically treated teeth were excluded from the study after visual and radiographic examination by an examiner.

Specimen preparation

Six teeth were sectioned longitudinally with a diamond disc in a slow-speed micromotor handpiece (NSK Ltd, Tokyo, Japan) which resulted in a total of twelve specimens. Each specimen was embedded in an acrylic block horizontally exposing the dentine surface. The dentine surfaces were grounded sequentially with 400, 500, 600, and 800 grit silicon carbide paper (CUMI, Carborundum Universal Limited, Chennai, India) followed by pumice polishing to obtain a smooth and even dentine surface.

Microhardness evaluation before exposure

Microhardness was measured for each sample before exposure to the test solutions. Microhardness was evaluated with a Vickers microhardness tester on the dentine surface approximately 0.5 mm from the root canal space using an indenter with a load of 200 g and a holding time of 10 seconds. The Vickers hardness number is then calculated from the indentations formed.

TABLE 3. Mean and standard deviation of the microhardness (VHN) values of the groups and descriptive statistics on comparison of microhardness reduction of the groups using One-way ANOVA test

Groups	n	Mean	Standard deviation	Standard error	p
Group C	6	16.6	6.764	2.762	0.113
Group D	6	10.517	5.275	2.153	

VHN: Vicker's hardness number

Randomisation

The specimens were then randomly divided into two groups,

- Group C – 17% EDTA (n=6)
- Group D – 16% Sodium gluconate (n=6)

Microhardness evaluation after exposure

The specimens were immersed in the corresponding test solution for five minutes. Microhardness was measured for each sample after exposure to the test solutions. The change in the microhardness was calculated as the difference between the baseline values and post-treatment values. The data were collected and tabulated for statistical analysis using SPSS software version 22.0 (IBM, USA) with a one-way ANOVA test.

RESULTS

The median, first quartile (Q1) and third quartile (Q3) values of the smear layer and those values of dentinal erosion of all the samples were listed in Table 1 and Table 2, respectively, and were analysed for statistical significance with Mann-Whitney U test using SPSS software version 22.0 (IBM, USA). The microhardness test of all the samples was done using a Vickers microhardness tester and the values were recorded in vicker's hardness number (VHN). The mean, standard deviation, and standard error were calculated. A parametric one-way ANOVA test was carried out using SPSS software version 22.0 (IBM, USA) to test the statistical significance ($p<0.05$) of the groups (Table 3). Figure 1 shows

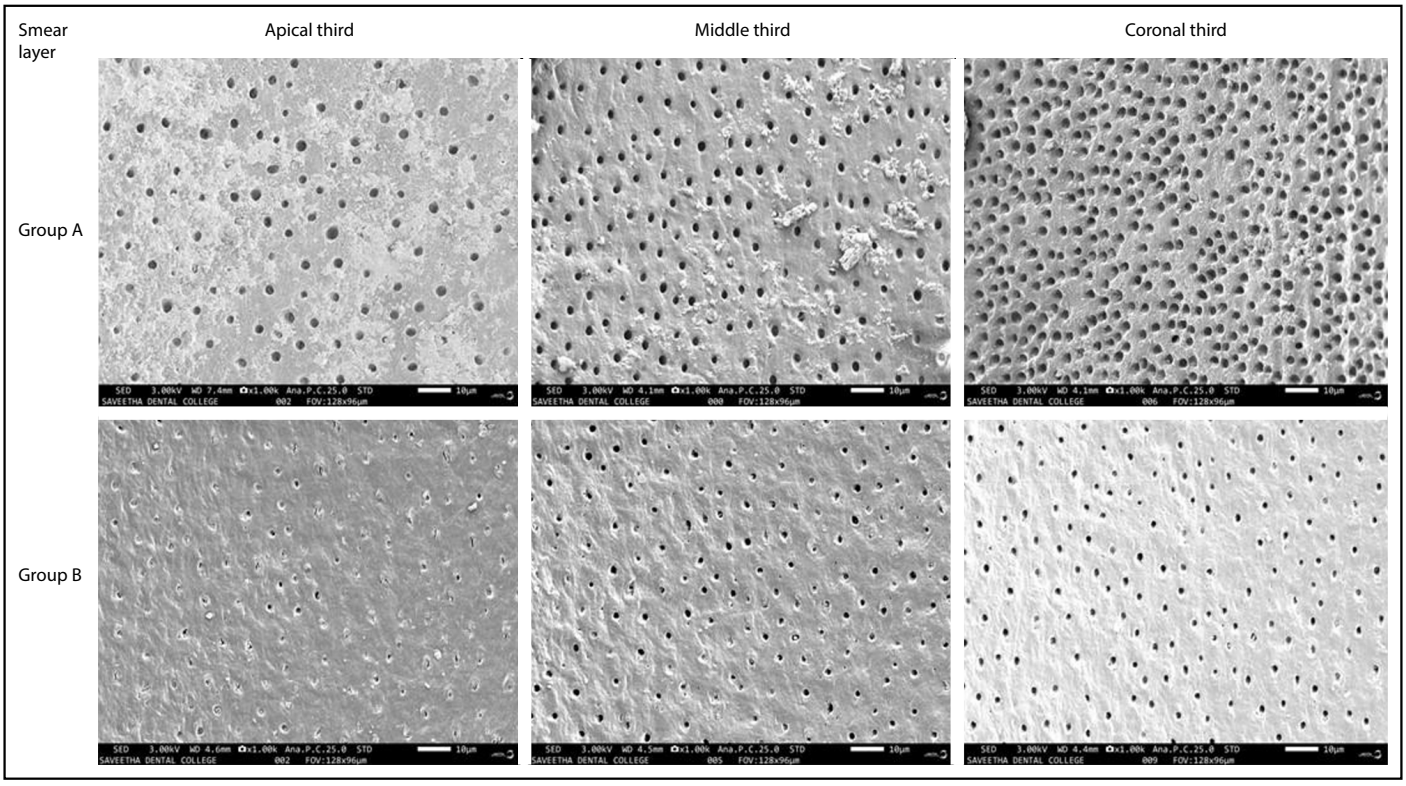

Figure 1. Representative images of the smear layer following final rinse in Group A and Group B at 1000x magnification

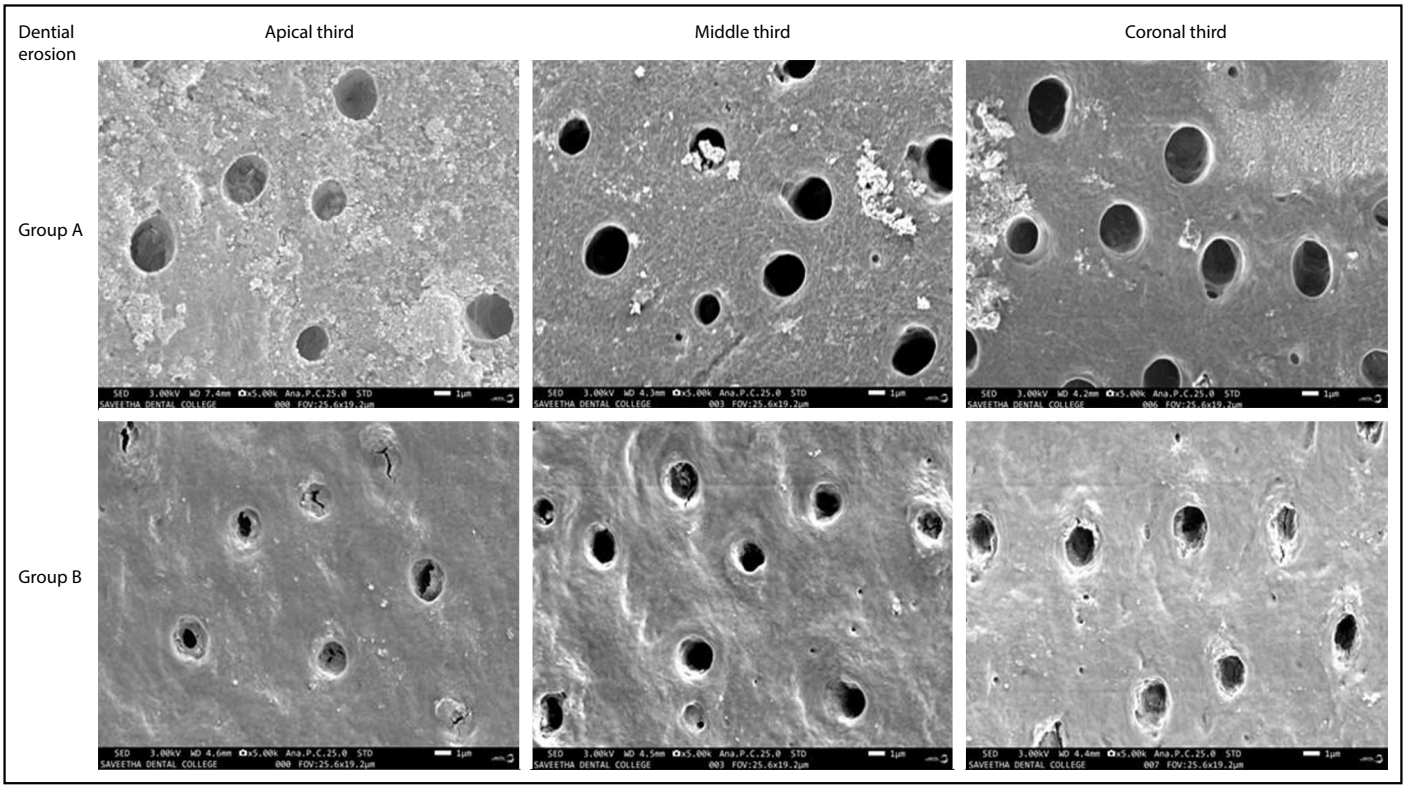

Figure 2. Representative images of dentinal erosion following final rinse in Group A and Group B at 5000x magnification

the representative SEM images of the smear layer following final rinse in Group A and Group B at 1000x magnification and Figure 2 shows the representative SEM images of dentinal erosion following final rinse in Group A and Group B at 5000x magnification.

Inference

Evaluation of smear layer removal

The smear layer removal ability of groups A and B was effective in the coronal and middle thirds of the root canals In group A and group B, the apical third showed a median score

of 2. However, the middle third and the coronal third in both groups showed a median score of 1. No significant difference was observed between the two groups.

Evaluation of dentinal erosion

The dentinal erosion in group A showed a median score of 1 in the apical third and a median score of 2 in both the coronal and the middle thirds whereas in group B, a median score of 1 was observed in the apical third, the coronal and the middle thirds. A statistically significant difference was observed between the two groups in the coronal (p=0.001) and the middle thirds (p=0.002).

Evaluation of microhardness

The dentine microhardness reduction in group C showed a mean score of 16.6 whereas in group D, a mean score of 10.5167. Group C showed a more decrease in microhardness compared to group D, however, statistical analysis using a one-way ANOVA test showed no statistically significant difference between the two groups (p=0.113).

DISCUSSION

The first and third null hypothesis was accepted as no statistically significant difference were present in smear layer removal efficacy and microhardness reduction. However the second null hypothesis was partially rejected as statistically significant difference were present in dentinal erosion in middle third and coronal third but not in the apical third of the root canal between the two groups.

The most commonly used chelating agent for smear layer removal is 17% EDTA (13) in conjunction with sodium hypochlorite, however, it causes significant dentinal erosion (7). Over the years, several irrigation solutions have been extensively examined to overcome the drawbacks associated with the use of EDTA. The quest for an irrigant with a controlled chelating activity led to the introduction of alternatives such as sodium gluconate, a derivative of gluconic acid (8). It is produced using submerged fermentation of glucose obtained from Zea mays with *Aspergillus niger* (8). It is a potent chelator whose chelating ability can be controlled by increasing or decreasing the pH of the solution (9). The parameters evaluated in this study were smear layer removal, dentinal erosion, and dentine microhardness reduction.

The results of the current study revealed that both the irrigants showed comparatively more smear removal in the coronal and the middle thirds compared to the apical third. This reduced smear layer removal in the apical third by EDTA is due to the increased surface tension and larger molecular size (14). The same reason could be attributed to sodium gluconate due to its similar molecular size (15). Sodium gluconate showed a marginally better smear layer removal than EDTA in the coronal third however, no statistically significant difference was noted. On the other hand, the smear layer removal capability of sodium gluconate was similar to EDTA in the middle and the apical thirds of the root.

On the contrary, dentinal erosion evaluation revealed that sodium gluconate exhibited significantly less dentinal erosion compared to EDTA in the coronal and the middle thirds. Whereas in the apical third, no significant difference was found in dentinal erosion between the two groups. The reason behind this could be attributed to the difference in the chelating activity between the two irrigating solutions.

The chelating agents form complexes by reaction of their negatively charged donor groups with polyvalent metal ions. These chelating agents can be the EDTA type and the aldonic type (e.g., sodium gluconate) (16). The choice of chelating agent depends upon the conditions under which it is intended. The EDTA is a hexadentate ligand as it binds to metals through four carboxylate and two amine groups (17). It is effective in acid, neutral and alkaline conditions (14).

On the other hand, sodium gluconate is a polyhydroxycarboxylic acid (18), and its chelating ability increases with an increase in the pH of the environment. The calcium-chelating ability of sodium gluconate is through the carboxylic oxygen atom and the α-hydroxylic ligand (19). It can chelate iron strongly in neutral conditions but bivalent metallic ions like calcium ions require a strongly alkaline pH. The high alkaline pH is essential to disengage protons from the hydroxyl groups, thereby creating anionic centres which are known to bind metals strongly (20).

In this study, sodium gluconate was employed at an optimum pH of 9 wherein the sodium gluconate selectively forms a calcium gluconate complex through the carboxylic oxygen atom alone as the anionic centres cannot be formed. This has led to the selected chelating ability of sodium gluconate to chelate calcium in an unorganised framework of the smear layer was therefore similar to EDTA (20), as no difference was observed in smear layer removal capability. However, with respect to chelating calcium from a well-organised calcium hydroxyapatite crystals framework, the sodium gluconate was not as aggressive as EDTA and produced significantly less dentinal erosion.

Furthermore, the dentine decalcification effect of the two irrigants was evaluated indirectly through microhardness evaluation, which revealed that the sodium gluconate was better than EDTA, as it produced comparatively less decrease in dentine microhardness; however, this difference in the activity between the two groups was not statistically significant.

The current study has limitations. Dentine is an anisotropic medium, and its characteristics vary with the change in the parameters under which it is tested. Another possible limitation could be that the study being an *in-vitro* study the volume of irrigant in a root canal is small compared to when used for immersing the specimen. However, the use of standardized circumstances allowed for comparable results between the two irrigants.

In the future, further *in vitro* and *in vivo* studies are needed to evaluate the efficacy of sodium gluconate based on its ability to remove calcium hydroxide from the canal walls, its antibacterial efficacy, and its ability to be used as a continuous chelating agent.

CONCLUSION

Within the limitations of the study, it can be concluded that smear layer removal capability of sodium gluconate is comparable to EDTA and both the irrigants were effective in the coronal and the middle third compared to the apical third. Sodium gluconate did not cause dentinal erosion, and produced a reduction in dentine microhardness which can be regulated by adjusting the pH of the solution. Sodium gluconate can achieve a balance between optimum smear layer removal and reduced dentine decalcification, hence, it can be considered a potential alternative as a final irrigant in place of EDTA.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by The Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai Ethics Committee (Date: 19/07/2022, Number: CSP/22/MAY/110/329).

Peer-review: Externally peer-reviewed.

Financial Disclosure: This study did not receive any financial support.

Authorship contributions: Concept – H.R.K., L.B., M.R.R., A.R.; Design – L.B., M.R.R., A.R.; Supervision – H.R.K., L.B., M.R.R., A.R.; Funding - H.R.K.; Materials - H.R.K.; Data collection and/or processing – H.R.K.; Analysis and/or interpretation – H.R.K., L.B., M.R.R., A.R.; Literature search – H.R.K.; Writing – H.R.K., L.B., M.R.R., A.R.; Critical Review – H.R.K., L.B., M.R.R., A.R.

REFERENCES

1. Azim AA, Griggs JA, Huang GTJ. The Tennessee study: factors affecting treatment outcome and healing time following nonsurgical root canal treatment. Int Endod J 2016; 49(1):6–16. [CrossRef]
2. Siqueira JF, Jr, Alves FR, Almeida BM, de Oliveira JC, Rôças IN. Ability of chemomechanical preparation with either rotary instruments or self-adjusting file to disinfect oval-shaped root canals. J Endod 2010; 36(11):1860–5. [CrossRef]
3. Chugal NM, Clive JM, Spangberg LSW. Endodontic infection: some biologic and treatment factors associated with outcome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 96(1):81–90. [CrossRef]
4. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94(6):658–66. [CrossRef]
5. 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. Braz Dent J 2012; 23(6):654–8. [CrossRef]
6. Poudyal S, Wei-Hong P. Effect of ethylenediaminetetraacetic Acid (EDTA) gel on removing smear layer of root canal *in vitro*. Chin Med Sci J 2012; 27(3):190–1. [CrossRef]
7. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int Endod J 2002; 35(11):934–9. [CrossRef]
8. Canete-Rodríguez AM, Santos-Duenas, IM, Jimenez-Hornero JE, Ehrenreich A, Liebl W, García-García I. Gluconic acid: properties, production methods and applications—an excellent opportunity for agro-industrial by-products and waste bio-valorization. Process Biochem 2016; 51(12):1891–1903. [CrossRef]
9. Abdelazim MH, Abdelazim AH. Effect of sodium gluconate on decreasing elevated nasal calcium and improving olfactory function post COVID-19 infection. Am J Rhinol Allergy 2022; 36(6):841–8. [CrossRef]
10. Rabie A, Nase-El-Din H. Sodium gluconate as a new environmentally iron chelating agent for HP/HT stimulation treatments. 19th Middle East Oil & Gas Show and Conference; March 2015; Manama, Kingdom of Bahrain. [CrossRef]
11. Rödig T, Hülsmann M, Kahlmeier C. Comparison of root canal preparation with two rotary NiTi instruments: Profile .04 and GT Rotary. Int Endod J 2007; 40(7):553–62. [CrossRef]
12. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of the smear layer. J Endod 2003; 29(3):170–5. [CrossRef]
13. Zehnder M, Schicht O, Sener B, Schmidlin P. Reducing surface tension in endodontic chelator solutions has no effect on their ability to remove calcium from instrumented root canals. J Endod 2005; 31(8):590–2. [CrossRef]
14. Arun DR, Sujatha V, Mahalaxmi S. Effect of 5% carbohydrate derived-fulvic acid on smear layer removal and root dentin microhardness - an *in vitro* study. Eur Endod J 2022; 7(2):156–60.
15. National Center for Biotechnology Information. PubChem Compound Summary for CID 23672301, Sodium gluconate. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-gluconate. Accessed June 22, 2023.
16. Scholnick F, Pfeffer PE. Iron chelating capacity of gluconamides and lactobionamides. J Dairy Sci 1980; 63(3):471–3. [CrossRef]
17. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. Eur J Dent 2013; 7(Suppl 1):S135–42. [CrossRef]
18. Abbadi A, Gotlieb KF, Meiberg JBM, Peters JA, van Bekkum H. New Ca-sequestering materials. Green Chem 1999; 1(5):231–5. [CrossRef]
19. Phadungath C, Metzger LE. Effect of sodium gluconate on the solubility of calcium lactate. J Dairy Sci 2011; 94(10):4843–9. [CrossRef]
20. Hodge JE, Nelson EC, Moy BF. Metal chelation by glucose-ammonia derivatives. J Agric Food Chem 1963; 11:126–9. [CrossRef]