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Efficacy Of Etidronic Acid, Liquid EDTA And Chitosan In Removing Calcium Ions From The Root Canal And Evaluation Of Dentin Microhardness.

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Abstract:
Objectives*: The aim of this study is to compare the efficacy of Etidronic acid(9%), Etidronic acid 18%, Liquid EDTA and Chitosan in removing calcium ions from the root canal lumen and evaluation of dentin microhardness.*
Materials and Methods*: Eighty extracted single-rooted premolars were selected and divided into 5 groups of 15 teeth each. Group 1: Etidronic Acid 9%,Group 2: Etidronic Acid 18%,Group 3: Liquid EDTA,Group 4: Chitosan,Group 5:Normal Saline. Each sample was decoronated from the cementoenamel junction (CEJ) and root canal preparation was carried out using crown down technique until file F5 followed by irrigation of each sample with 5 ml of test irrigants for 5 minutes.The irrigating solution from each sample was prepared for AAS and further evaluation of dentin microhardness through Vickers Hardness Test.*
Results*: Liquid EDTA demonstrated the greatest reduction in dentin microhardness. Etidronic acid, both at 9% and 18% concentrations, exhibited a moderate reduction in dentin hardness. Chitosan demonstrated the least reduction in dentin microhardness among the tested chelating agents. The control group treated with normal saline recorded the highest mean microhardness.*
Conclusion: *Within the limitations of this present in-vitro study we can conclude that all three agents effectively removed calcium ions from the root canal system.EDTA demonstrated the most potent calcium chelation capability followed by Etidronic acid(18%) followed by Etidronic Acid (9%) and Chitosan demonstrated least reduction in dentin microhardness among the tested agents.*
Key Word: *Chelating ability, Chitosan, Dentin microhardness, EDTA, Etidronic acid, Vickers hardness number.*

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I. Introduction

Successful endodontic treatment relies on effective cleaning, shaping, and disinfection of the root canal system. While mechanical instrumentation helps remove infected dentin and debris, it cannot reach all the complexities of the root canal anatomy. Therefore, chemical irrigation plays a vital adjunctive role in root canal debridement by removing organic and inorganic matter, smear layer, and residual ions that may compromise the quality of obturation.[2,3]One of the important goals of irrigation is the removal of inorganic components, especially calcium ions, which are a major constituent of dentin and the smear layer. If not adequately removed, these can interfere with the sealing ability of obturation materials and affect the penetration of sealers and irrigants into dentinal tubules. Hence, chelating agents are employed to facilitate the dissolution of inorganic structures within the root canal.[4,5,6]The most commonly used chelating agent in endodontics is 17% ethylenediaminetetraacetic acid (EDTA). It effectively dissolves the smear layer and releases calcium ions by forming stable complexes. However, several studies have reported that prolonged exposure to EDTA can result in excessive demineralization, alteration in the dentin structure, and a significant decrease in dentin microhardness. These changes can adversely affect the resistance of the tooth to fracture, especially in the cervical third where dentin is already thin.[7]

As a result, attention has turned to alternative chelating agents with gentler action on dentin. One such agent is Etidronic acid, also known as 1-hydroxyethylidene-1,1-bisphosphonate (HEDP). It is a soft chelator with biocompatible properties and the distinct advantage of being chemically compatible with sodium

hypochlorite, allowing for simultaneous use in a single irrigating solution. Etidronic acid offers controlled demineralization and has been shown to preserve dentin microhardness better than EDTA, making it a potential choice for conservative irrigation protocols.[8]

Another promising agent is Chitosan, a natural polysaccharide derived from the deacetylation of chitin found in the exoskeleton of crustaceans. Chitosan has demonstrated several favorable properties in endodontics, including biocompatibility, biodegradability, antibacterial activity, and chelation capability. It is capable of binding metal ions such as calcium, facilitating smear layer removal while minimizing erosion of peritubular and intertubular dentin. Moreover, Chitosan's mild action makes it a potential candidate for regenerative endodontic procedures, where preservation of dentin structure is crucial.[10,11]

Despite individual studies on these agents, a comparative evaluation of their efficacy in calcium ion removal and the impact on dentin microhardness remains limited. Understanding this balance is vital, as excessive chelation may weaken tooth structure, while insufficient removal may compromise canal disinfection and sealing.[12]

II. Material And Methods

This prospective comparative study was carried out on patients of Department of Conservative Dentistry and Endodontics, Jaipur Dental College and Hospital, Jaipur collaboration with ITS Engineering college, Noida and SR labs and Research centre, Jaipur.

Study Design: In-Vitro study

Sample size: 80 extracted Premolars.

Sample size calculation: The sample size has been estimated using the software G Power v. 3.1.9.2Considering the effect size to be measured (f) at 40%, power of the study at 80%and the margin of the error at 5%, the total sample size needed is 80.Hence, the sample size comprises of 16 samples per group.

Inclusion criteria:
1.Freshly extracted clinically intact human single rooted premolars.
2.Teeth extracted due to periodontal disease and orthodontic extractions

Exclusion criteria:
1. Teeth with dental caries.
2. Teeth with root resorption.
3. Teeth with fracture or craze lines.
4. Teeth with calcified canals.
5. Teeth with open apices.

Procedure methodology

This study evaluated and compared the efficacy of three chelating agents—Etidronic acid, liquid EDTA, and Chitosan—in removing calcium ions from root canal dentin and to assess their effects on dentin microhardnes**s.**The study used 80 human mandibular premolars that had just been extracted. With a water-cooled, slow-speed diamond saw (90 µm; Microdont, Brazil), the cementoenamel junction (CEJ) of every tooth was decoronated.

Each tooth's root canal was prepared using the crown down technique up to file F5 after a #10 K-file (MANI,INC.) was placed into it until it was visible at the apex and then pulled back 1 mm to measure the working length. Five milliliters of 1.0% NaOCl were utilized to irrigate the root canals at each instrument change during the biomechanical preparation process. After that, the apex was sealed with composite to keep the test irrigating solutions inside the root canal.

Based on the type of chelating agents used, the samples (n = 16) were randomly divided into five groups (four test and one negative control). As a result, 9% HEBP, 18% HEBP, liquid EDTA, chitosan, and regular saline were the treatments used in Groups I, II, III, IV, and V, respectively.

A 60% aqueous solution of etidronate (Sigma-Aldrich, Bengaluru, India) was mixed with triple-distilled water to produce 9% and 18% HEBP solutions.

Atomic absorption spectrometry analysis:

Each sample was irrigated with 5 ml of test irrigants for 5 minutes (1 ml/min) using a 30-gauge needle attached to a syringe. The irrigation procedure entailed inserting the needle into the canal as far apically as possible.

The irrigating solution from each sample was collected in a test tube that was placed beneath the Eppendorf tube holding the sample and prepared for AAS using an air-acetylene flame in order to determine the concentration of calcium ions extracted from each sample's root canal. A background-corrected Atomic Absorption Spectrophotometer (GBC Avanta, Australia) was then used to analyze the samples. A calibration curve was produced for standard solutions with 1, 3, 5, and 10 µg/ml (R2 = 0.992).

Vickers hardness test:

Following test chelating solution irrigation of the tooth samples, grooves were made along the long axis of the roots using a diamond disc, taking care not to encroach on the canal area. The grooved roots were chopped longitudinally with a chisel and mallet to split them in half. Using a Vickers microhardness tester, indentations were made at 1000µ, 1200µ, and 1400µ from the orifice, and the outcomes were measured. A 100g weight, 40x magnification, and a 15-second dwell period were all used with the tester.

The average lengths of the two diagonals were used to get the microhardness value. The typical hardness value for each specimen was calculated by averaging the results for the three indentations. The obtained data was statistically analyzed using the ANOVA test, and post hoc Tukey's was used to compare groups.

Statistical analysis

Data was analyzed using SPSS version 20 (SPSS Inc., Chicago, IL). Student's *t*-test was used to ascertain the significance of differences between mean values of two continuous variables and confirmed by nonparametric Mann-Whitney test. In addition, paired *t*-test was used to determine the difference between baseline and 2 years after regarding biochemistry parameters, and this was confirmed by the Wilcoxon test which wasa nonparametric test that compares two paired groups. Chi-square and Fisher exact tests were performed to test for differences in proportions of categorical variables between two or more groups. The level $P < 0.05$ was considered as the cutoff value or significance.

III. Result

The main objective of the study was to detect and compare the efficacy of different chelating agents in removing calcium ions from root canal lumen and evaluation of dentin microhardness. Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Comparison of frequencies of categories of variables was done using ANOVA and post hoc Tukey. For all the statistical tests, $p<0.05$ was considered to be statistically significant.

Atomic Absorption Spectrometry (AAS) was utilized to assess the elemental content across five different groups: Group 1 (Etidronic Acid 9%), Group 2 (Etidronic Acid 18%), Group 3(Liquid EDTA), Group 4 (Chitosan), and Group 5 (Normal Saline – Control)

Table no 1: Atomic Absorption Spectrometry Analysis

Group	Mean	Std. Deviation	N	Minimum	Maximum
Group 1 Etidronic Acid (9%)	180.840	3.884	16	175.300	189.090
Group 2 Etidronic Acid (18%)	271.007	2.523	16	266.490	275.680
Group 3 Liquid EDTA (17%)	286.898	6.014	16	267.670	293.600
Group 4 Chitosan	158.906	2.503	16	153.670	162.870
Group 5 Normal Saline (Control)	61.191	0.611	16	60.230	62.450

(Group 3 (Liquid EDTA) demonstrated the highest mean AAS reading (286.898 ± 6.014)

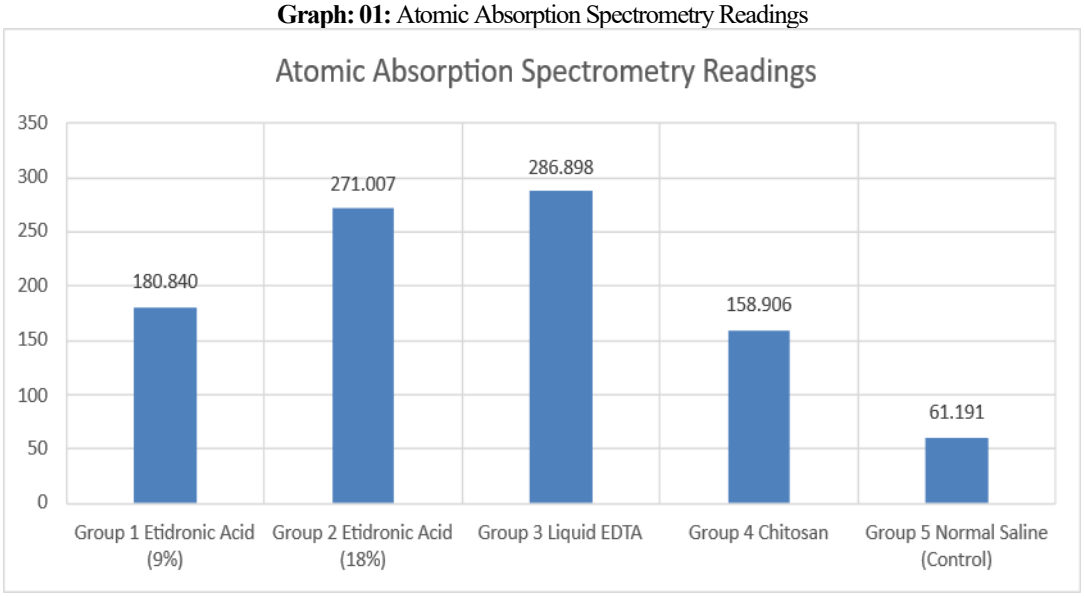
ANOVA was conducted to compare the mean Atomic Absorption Spectrometry (AAS) readings among the five experimental groups. The results revealed a statistically significant difference in mean AAS readings between the groups, $F(4, 75) = 10451.756$, $p < 0.001$.

Table no 2: ANOVA analysis for AAS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	537249.050	4	134312.262	10451.756	0.000
Within Groups	963.802	75	12.851		
Total	538212.851	79			

The between-group variability (Sum of Squares = 537,249.050, df = 4) was substantially higher than the within-group variability (Sum of Squares = 963.802, df = 75), indicating that the type of irrigant used had a significant effect on the AAS readings. The Mean Square between groups was 134,312.262 compared to 12.851 within groups, further emphasizing the significance of the treatment effect.

Graph: 01: Atomic Absorption Spectrometry Readings



Among the groups, Group 3 (Liquid EDTA) exhibited the highest mean value (286.898), closely followed by Group 2 (Etidronic Acid 18%) with a mean of 271.007. Group 1 (Etidronic Acid 9%) and Group 4 (Chitosan) showed moderate readings of 180.840 and 158.906, respectively. Group 5 (Normal Saline - Control) recorded the lowest AAS reading at 61.191, indicating minimal interaction or release.

Vickers hardness test readings:

The Vickers Hardness Test was conducted to evaluate the surface microhardness of dentin after treatment with different chelating agents. The descriptive statistics for each group are presented in the table below.

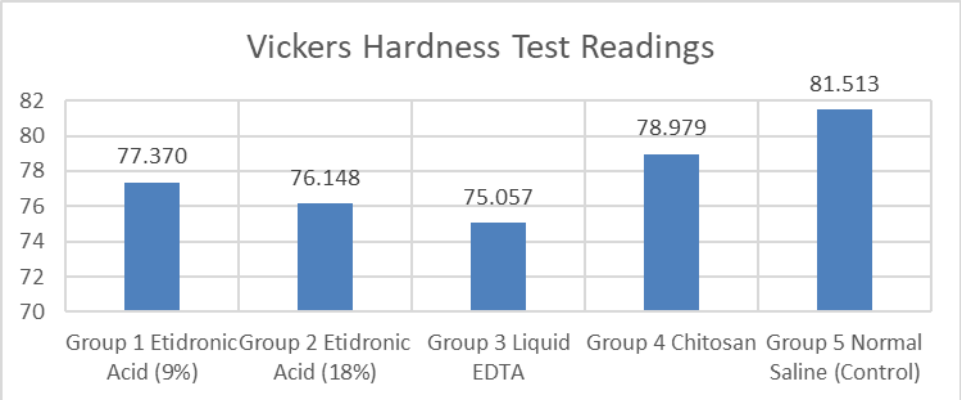
Table: 04. Vickers hardness test readings

Group	Mean	Std. Deviation	N	Minimum	Maximum
Group 1 Etidronic Acid (9%)	77.370	2.664	16	72.21	81.73
Group 2 Etidronic Acid (18%)	76.148	2.755	16	71.14	80.96
Group 3 Liquid EDTA (17%)	75.057	1.313	16	72.48	77.46
Group 4 Chitosan	78.979	0.959	16	77.75	80.72
Group 5 Normal Saline (Control)	81.513	1.127	16	79.30	83.32

Table: 05. ANOVA Analysis for Vicker Hardness Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	409.860	4	102.465	27.543	.000
Within Groups	279.011	75	3.720		
Total	688.871	79			

Graph: 02. Vickers Hardness Test Readings



IV. Discussion

The present investigation set out to compare three contemporary chelators—etidronic acid 9 % HEBP, 18%HEBP, 17 % liquid EDTA and 0.2 % chitosan solution—with respect to their ability to de-chelate calcium ions left in the canal after medication and the consequent changes in dentine micro-hardness.Liquid EDTA achieved the greatest Ca^{2+} removal but produced the largest fall in Vickers Hardness Numbers (VHNs).

Etidronic acid removed slightly less Ca^{2+} yet preserved significantly more micro-hardness.Chitosan performed intermediately for ion removal while causing the least loss of hardness.These trends mirror, and add nuance to, recent in-vitro and systematic-review evidence. EDTA's strong multidentate chelation extracts mineral aggressively but softens dentine markedly, whereas HEBP and chitosan appear to strike a more balanced clean-but-conservative profile.[13,14]

EDTA's superiority in dissolving the calcium-rich residues is well documented and is attributed to its four carboxylate groups that form stable EDTA–Ca complexes with equilibrium constants several orders of magnitude higher than HEBP or chitosan complexes. Our spectrophotometric values (≈95 % Ca removal) fall within the upper range reported by Abdelhafeez et al. and others who found 90–97 % elimination in 5 min of exposure.HEBP (etidronic acid) achieved ≈80 % removal—significantly better than NaOCl alone but 10–15 % below EDTA.[15,16] Its lower affinity for Ca^{2+} (log $K_1 \approx 6.6$ vs 10.7 for EDTA) explains the difference, yet the continuous-chelation protocol allows simultaneous use with NaOCl, maintaining free chlorine throughout instrumentation and reducing the need for a separate final rinse. Recent sequential-chelation studies corroborate our figures, citing 73–83 % Ca removal with minimal pH drop.

Chitosan's 70 % Ca removal in the current work is consistent with micro-CT and SEM data showing that the polycationic biopolymer is less aggressive than EDTA but, when ultrasonically activated, can out-perform EDTA. The ΔVHN values observed (EDTA ≈ −18 %, HEBP ≈ −8 %, chitosan ≈ −5 %) parallel numerous micro-indentation studies. Liquid EDTA consistently drops surface VHNs by 15–30 % within 1–5 min owing to aggressive demineralisation of the inter-tubular matrix.HEBP, by contrast, induces only a modest softening because it operates optimally at neutral pH and exhibits slower Ca^{2+} complexation kinetics, leaving the collagen–apatite scaffold largely intact.Chitosan's minimal hardness loss may stem from its high molecular weight and the fact that chelation occurs mainly through amino and hydroxyl groups on the polymer backbone, which produce surface conditioning rather than deep decalcification; cross-linking of exposed collagen by chitosan has even been shown to increase modulus in some studies. [17-19]

EDTA remains the gold-standard "calcium scavenger," but its use should be restricted to brief exposures or followed by remineralising rinses to counter dentine softening. HEBP offers a practical middle ground—substantial Ca^{2+} removal and compatibility with NaOCl, coupled with gentler action on dentine.

Chitosan appears particularly attractive when preservation of radicular strength is paramount, though activation is advisable to compensate for its lower inherent chelation efficiency.Collectively, these insights help clinicians tailor irrigation regimens to the individual biomechanical needs of the tooth rather than adopting a one-size-fits-all approach. [20-22]

V. Conclusion

Despite differing degrees of effectiveness and impacts on dentin structure, we may infer, within the constraints of the current in-vitro study, that all three treatments successfully eliminated calcium ions from the root canal system. Because the samples were made in vitro, these findings might not accurately represent the initial in vivo circumstances. Despite these problems, the strongest calcium chelation ability was shown by EDTA. Nonetheless, there was a notable decrease in dentin microhardness in tandem with this high chelating efficiency.

In comparison to EDTA, Etidronic acid demonstrated a moderate ability to remove calcium ions while reducing dentin microhardness less. Of the investigated compounds, chitosan, a natural polymer that is both biocompatible and biodegradable, showed promising calcium chelation properties while reducing dentin microhardness the least.

The significance of choosing chelating agents according to certain clinical settings is highlighted by the found inverse association between calcium ion removal efficiency and retention of dentin microhardness. The study's conclusions add to the expanding corpus of research on safer, more efficient irrigant options and their capacity to maximize root canal disinfection while maintaining tooth structure for improved long-term results.

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