Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 july; 6(7s): 1155-1162

Comparative Evaluation of 17% Ethylenediaminetetraacetic acid and 9 % 1-Hydroxyethylidene-1, 1-Bisphosphonate on the Fracture Resistance of Endodontically Treated Teeth with Varying Dentin Thickness – an In-Vitro Study

Dr. Shreya Agarwal[1], Dr Sandhya Kapoor Punia[2*], Dr. Yogender Kumar[3], Dr. Shilpi Kushwaha[4], Dr. Divyanshu Choudhary[5]

[1]Affiliation-Post Graduate, Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Udaipur, India , Mail id- shreyaagarwal755@gmail.com , Orchid id - 0009-0005-2558-799X
Contact No. - +91-9588049154
[2*]Affiliation- Professor and Head of Department, Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Udaipur, India , Mail Id- drsanvikpunia@gmail.com, Orchid id – 0009-0004-1765-8412, Contact No. - +91- 9660987078
[3]Affiliation – Professor, Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Udaipur, India Mail id - dr.yogi09@gmail.com. Orcid id. - 0009-0002-4699-3792, Contact No. - +91-9828206725
[4]Affiliation – Senior Lecturer, Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Udaipur, India , Mail id -drshilpi.endo@gmail.com, Orcid id - 0009-0002-9034-7261,Contact No-+91-7983078790
[5]Affliation - Post Graduate, Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Udaipur, India, Mail id-drdivyanshuchaudhaty@gmail.com , Orchid id – 0009-0007-2938-4529
Contact No.- 7597858931

***Corresponding author –** Dr Sandhya Kapoor Punia
*Professor and Head of Department, Department of Conservative Dentistry and Endodontics,Darshan Dental College and Hospital, Udaipur, India , Mail Id-drsanvikpunia@gmail.comOrchid id – 0009-0004-1765-8412,Contact No. -+91-9660987078

Abstract

Aims & Background: This study determine and compare the effects of 17% EDTA and 9% HEBP on the fracture resistance of endodontically treated teeth with different dentin thicknesses, focusing on how these chelating agents, which demineralize tooth tissues, impact structural integrity.
Materials and Methods: Thirty extracted premolars with single canal were selected. Decoronation was performed to maintain a root length of 13 mm. Working length was established, and canal preparation was carried out using ProTaper Universal files up to size F3 under copious irrigation. Using CBCT, specimens with remaining dentin thickness between 1.75 mm and 2.00 mm were chosen. The specimens were divided into three groups: Group A- 17% EDTA and Group B-9% HEBP. The prepared canals were irrigated with assigned chelating agent (9 ml, 3 minutes) and obturated using AH Plus sealer, and GuttaPercha. The specimens were then placed in acrylic blocks coated with polyvinyl siloxane, exposing 4 mm of root. Fracture resistance was assessed using a universal testing machine. The results were tabulated and statistically analyzed using one-way ANOVA.
Results: The mean fracture resistance was greatest in Group B (956.000 ± 206.596 N), with Group A following (802.267 ± 161.179 N).
Conclusion: The 9% HEBP had higher mean fracture resistance, pointing to its potential as a more effective chelating agent than 17% EDTA and normal saline.
Clinical significance: Etidronic acid, Fracture resistance, EDTA

Introduction:

Root canal treatment is a pivotal procedure in contemporary dentistry, aimed at addressing endodontic diseases by thoroughly eliminating microorganisms and cleaning the infected root canal system. The precise chemomechanical debridement of the canal, which combines chemical irrigation with mechanical instruments, is essential to the success of root canal therapy.[1, 2]
Since Nygaard-Ostby first introduced chelating compounds in Endodontics in 1957, their function has been thoroughly studied. Chelating agents are defined by their capacity to combine with metal ions to create stable complexes, which helps the teeth's hard tissues demineralize.[3] The inorganic component of the smear layer, which is a result of mechanical

instrumentation and can impede the passage of sealers and irrigants into the dentinal tubules, is disrupted by this demineralization.[4]
The two chelating agents that are the subject of this study, 9% HEBP and 17% EDTA, both have unique features and modes of action that affect both their efficacy and the dentin's structural integrity. While HEBP is renowned for its milder action, EDTA is known for its strong chelating capabilities.[5, 6]
Chelating agents are essential for root canal treatment for a number of reasons, including: removal of the smear layer, improvement in disinfection, adherence improvement and dentin structure preservation.[4] Nevertheless, the physical characteristics of dentin may change as a result of chelating agents' contact with dentin. They can weaken the dentin, increasing its vulnerability to fracture, even as they help disinfect and clean the root canal.[7]
EDTA was introduced to Endodontics in the mid-20th century and has since become one of the most commonly used chelating agents. Its ability to form complexes with calcium ions in dentin has made it a valuable tool for root canal preparation. It is particularly efficient in demineralizing dentin and removing the smear layer created during mechanical instrumentation. However, its aggressive chelating action can lead to excessive demineralization, weakening the dentin structure. It functions by chelating calcium ions from the hydroxyapatite in dentin. This chelation process effectively removes the inorganic component of the smear layer, facilitating better access for irrigating solutions and obturating materials. The aggressive nature of EDTA, however, means that prolonged exposure can result in significant erosion of the dentin surface.[7, 8]
Several studies have documented the effects of EDTA on dentin. For instance, Calt and Serper (2002) demonstrated that prolonged exposure to EDTA can cause significant erosion of the dentin surface, leading to a decrease in microhardness and increased susceptibility to fracture. It occurs mainly because of its ability to chelate calcium ions, essential for maintaining the rigidity and strength of dentin. [7]
HEBP, also known as etidronic acid, is a bisphosphonate and a milder chelating agent compared to EDTA. Its primary use in medicine is to inhibit bone resorption, but it has found applications in Endodontics due to its ability to chelate calcium ions without significantly altering the dentin structure.[9]
It's important to discover the optimal chelating agent for endodontic therapy that maintains the mechanical integrity of dentin tissue while providing sufficient chelating activity. Therefore, the purpose of the current study was to assess how two distinct irrigants affected the roots' ability to withstand fracture.
This study's main objective was to evaluate and compare the effects of 9% HEBP and 17% EDTA on the fracture resistance of teeth that had received endodontic treatment. Despite being commonly utilized because of its strong chelating abilities, EDTA has a number of drawbacks, such as dentin erosion and denaturation of collagen fibrils. These consequences may weaken the dentin's microhardness and jeopardize the tooth's structural stability.[8]
In contrast, because it has less effect on the physical characteristics of dentin than EDTA, HEBP, a weaker chelator, is suggested as a workable substitute.[6] With a focus on how these two chelating chemicals affect treated teeth's fracture resistance which is a crucial factor in the long-term efficacy of endodontic treatment, this study seeks to distinguish between them.
The null hypothesis of this study was that there is no effect of different chelating agents on fracture resistance of endodontically treated teeth.

Methods:

This in-vitro study was conducted at the Department of Conservative Dentistry & Endodontics, Darshan Dental College and Hospital, Udaipur (Raj.) and Rahul Engineers Laboratory, Udaipur (Raj.) to compare the influence of 17% EDTA and 9% HEBP on fracture resistance of endodontically treated teeth with varying dentin thickness.
Thirty human premolars that were extracted for periodontal or orthodontic purposes were used in this study. The teeth were acquired from Darshan Dental College and Hospital in Loyara, Udaipur, specifically from the Department of Oral and Maxillofacial Surgery. Sound, intact, single-rooted tooth with single canal was one of the selection criterion. Using an ultrasonic scaler, all specimens were cleared of any deposits, debris, and calculus before being kept at room temperature in 0.9% normal saline. A stereomicroscope was used to examine every tooth to make sure there were no fractures, cracks, or additional defects.
To ensure uniformity and reliability in the results, strict inclusion and exclusion criteria were followed. Only teeth free from caries, restorations, cracks, root resorptions, immature apex, non- carious cervical lesions were included. Teeth with complex root canal systems, multiple canals, or previous endodontic treatment were excluded from the study.
To standardize the length of the specimens, all teeth were decoronated near the CEJ using a slow-speed diamond disc under water coolant to allow a standardized root length of 13 mm from the apex, ensuring uniformity across all specimens.
The working length of each root canal was determined by inserting a #15 K stainless steel file into the canal until it was visible at the apical foramen. The file was then retracted by 0.5 mm, and the working length was confirmed radiographically. Canal preparation was performed using hand ProTaper Universal files up to size F3. The use of 3%

NaOCl for 60 seconds and 0.9% Normal Saline for 60 seconds alternatively with normal saline as the final irrigant was aimed at optimizing disinfection while minimizing the risk of excessive demineralization.
Following canal preparation, the remaining dentin thickness of each specimen was evaluated using CBCT. The CBCT evaluation was performed using a high-resolution scanner, ensuring precise measurements of the remaining dentin thickness. Measurements were taken at three levels (3 mm, 7 mm, and 9 mm from the coronal end) and four sides (Buccal, Lingual, Mesial, and Distal). The standardized levels of 3 mm, 7 mm, and 9 mm were chosen to represent critical areas of the root that are commonly subjected to stress during function. The average remaining dentin thickness for each specimen was calculated using the formula:
M1+L1+B1+D1 = X1, M2+L2+B2+D2 = X2, M3+L3+B3+D3 = X3, X1+X2+X3/ 3 = Remaining Dentin Thickness. Only teeth with an average dentin thickness between 1.75 mm and 2.00 mm were included in the study to ensure uniformity and comparability (Fig. 1).
The average dentin thickness was calculated to ensure a consistent baseline for evaluating the effects of the chelating agents.
The specimens were then divided into two groups (n=15). Group A was irrigated with 17% EDTA, while Group B was irrigated with 9% HEBP (Preparation: HEBP was purchased from a commercial source and mixed with distilled water to a weight/volume ratio of 9%.) (Fig. 2).
A 31 gauge side vented needle with a stopper marked 1 mm short of the working length was used to manually irrigate the root canals after they had been prepared for canal sealing. 3 ml of the appropriate chelating agent was used in the irrigation process. The irrigant was then activated in the canal for 1 minute, 2 mm less than the working length, using an Endoactivator in cyclic axial motion with the 15/.02 and 22 mm tip running at the fastest speed recommended by the manufacturer. This procedure was repeated three times. The experimental solutions had a total irrigation volume of 9 ml for 3 min. Finally, distilled water was used to cleanse the root canals in order to prevent the long-term effects of the irrigants.
Following that, prepared root canals were dried with sterile paper points, obturated with AH Plus sealer and gutta-percha cones using single cone obturation method. 1 mm below the root canal access, excess obturation material was removed and vertically compacted. Root canal opening was then sealed with Type 2 GIC followed by a thin layer of cocoa butter. After that, the specimens were kept in an incubator at 37°C and 100% humidity for 24 hours to allow the complete setting of obturation and restorative material.
Following incubation, a cellophane sheet was placed over the apical 9 mm of each root to allow 1 mm of space for the impression material comprised of polyvinyl siloxane, which would simulate the periodontal membrane. The 4 mm coronal portions of the covered roots were then exposed when they were vertically inserted in self-curing acrylic resin blocks. The specimens with the cellophane sheets were taken out of the acrylic blocks after the acrylic had set. After the cellophane film was removed, polyvinyl siloxane impression material was used to line the acrylic block. In order to simulate the periodontal ligament in the mounted acrylic, the specimens without the cellophane sheet was reinserted into the polyvinyl siloxane coated acrylic blocks to a length of 9 mm. This resulted in a thin layer of impression material lining the root surface. The excess polyvinyl siloxane impression material was cut using B.P. blade.
The mounted specimens were then evaluated for fracture resistance using a universal testing apparatus after a 24-hour period. The force exerted was parallel to the specimen's long axis because of the vertical mounting of the acrylic blocks in the lower plate of the universal testing apparatus. The universal testing machine has a maximum load cell capacity of 2500 N. Using spherical steel balls with a diameter of 6 mm, a vertical compressive loading was applied at a rate of 1 mm/min in the region that corresponded to the specimen's center. Newtons were used to record the greatest stress required to fracture the specimen. Every value that was acquired was examined statistically.

Results:

The collected data was organized into a table and subjected to statistical analysis. The statistical analysis was performed using SPSS software (version 25.0). Descriptive statistics, including mean and standard deviation, were calculated for each group (Table 1). Descriptive and comparative statistics were employed to compare and demonstrate the findings, with a significance level (p) of 0.05. Any value equal to or less than 0.05 was deemed to be significant. The fracture resistance was assessed using One-way ANOVA.

Mean Fracture Resistance
The ANOVA analysis demonstrated a notable variance in fracture resistance across the groups, supported by the remarkably significant F-statistic ($F = 82.801$, $p < .05$). This implies a statistically significant distinction in fracture resistance values among both groups, prompting a deeper exploration into the unique group differences (Table 2).
The group exhibiting the highest average was determined to be 9% HEBP (956.000 ± 206.596 N), with the subsequent highest being 17% EDTA (802.267 ± 161.179 N) (Fig. 3).

Discussion:

In RCT, a variety of chelating agents have been used, such as chitosan, citric acid, EDTA, peracetic acid, etidronic acid, and maelic acid.[4]
The most popular application for EDTA is as an irrigant; however, a research by Zehnder found that EDTA had a number of drawbacks, including denaturation of collagen fibrils and peritubular and intertubular dentin erosion when used for more than three minutes. The root canal's adhesive properties may be impacted by these mineral changes in the dentin, which might harm root canal sealing.[8] In addition, Pawlicka H. claimed that EDTA significantly lowers dentin microhardness, especially in the dentin layer surrounding the root canal lumen which highlights the need of suitable replacement for EDTA.[5]
This study was done in- vitro as it is essential to assess systems in an in vitro model before transferring them into an in vivo setting in order to find treatments or materials that could improve clinical performances. Furthermore, extracted human teeth are used in a large amount of in vitro research on fracture resistance testing. The extracted teeth should be standardized in order to guarantee that the findings of the study are unaffected. The maxillary and mandibular premolars used in the current study were single rooted and single canal in order to minimize anatomical heterogeneity.[10]
All teeth were decoronated near CEJ to maintain a standard length of 13 mm from the apex, which was in accordance to the study done by Ayranci F et al. This was done to rule out the possibility that clinical crowns with varying dimensions would have an impact on the fracture strength results.[11]
Determining the working length precisely is essential during endodontic therapy. In this study, the genuine clinical working length (file tip at the level of the coronal most border of the main foramen) was calculated by subtracting 0.5 mm from the actual canal length. Williams et al. state that this approach is trustworthy.[12] To ascertain the radiographic working length, digital periapical radiographs were supplemented to this.
In this study, the No. 15 stainless steel K file was used as an endodontic instrument to measure working length. M.A. Martínez-Lozano et al. carried out a pilot study using multiple endodontic files to determine which file offers the most precise measurements. They recommended utilizing a no. 15 K file to ascertain the working length of single-rooted teeth.[13]
A Protaper hand file system based on NiTi was employed in this investigation. Its features include a convex triangular cross-section, positive-angled, sharp cutting edges, progressive taper radial lands, an advanced U-shaped flute design for increased flexibility, variable taper, rake angle that is more positive along the instrument's length, and a pitch-helix angle balanced to keep the instrument from screwing into the canal. When compared to the Mtwo and WaveOne file systems, the hand ProTaper file system showed the least reduction in dentin thickness, according to a research by Nidhi Rathi et al.[1]
Retaining the native canal geometry with less invasive techniques is associated with better endodontic outcomes. A well-prepared root canal should be conical in shape, tapering gradually while preserving the canal's natural curve and preventing any transportation. The thickness of the dentin that remains following intraradicular procedures is the most important iatrogenic factor that seems to be connected to the root's susceptibility to fracture to incoming stress.[1] According to Ulusoy et al.'s study from 2021, the amount of dentine thickness that remained enhanced the fracture resistance. This result was in line with other studies that have demonstrated that maintaining healthy dentine is one of the key factors affecting how long teeth that have had endodontic treatment will last.[11]
Thus, the current study used uniform root canal preparations. Each specimen's residual dentin thickness was also determined with the help of CBCT. The specimens that had dentin thickness remaining in the range of 1.75 mm to 2.00 mm were chosen.
Since 17% EDTA is the most widely used chelating agent was utilized as the test material for Group A in this study. This amino acid is often used to sequester divalent and trivalent metal ions. When EDTA is mixed with Mn (II), Cu (II), Fe (III), and Co (III), powerful compounds are produced. The primary elements in its manufacture are formaldehyde, sodium cyanide, 1, 2-diaminoethane (ethylene diamine), and water. Tetrasodium salt is created as a result, and it may be acidified to create the acidic forms. It is useful as a chelating agent and hexadentate ligand due to its ability to sequester metal ions such as calcium and iron. However, it demineralizes the inorganic smear layer components via calcium chelation. It binds with the calcium ions in hydroxyapatite crystals to release them from the dentin by forming stable, water-soluble compounds. This process may result in changes to the microstructure of the dentin and the calcium to phosphorus ratio.[7]
This study was conducted to explore alternative chelating agents that could address the limitations of EDTA and provide enhanced properties for radicular dentin. In Group B, 9% HEBP was utilized as the test material. HEBP, also known as 1-hydroxyethylidene-1,1-bisphosphonate, etidronate, or etidronic acid, was found to have no adverse effects. Research by De – Deus et al revealed that it takes 300 seconds for HEBP solutions to completely remove the smear layer.[14] Additionally, Zehnder et al noted that sodium hypochlorite and tetra sodium HEBP salt are highly compatible, recommending the use of HEBP due to its lack of short-term reactivity with NaOCl. As a result, HEBP has been proposed as a potential substitute for EDTA.[15]

In accordance with Kandaswamy et al., the demineralization kinetics induced by 9% HEBP were significantly slower compared to 17% EDTA, resulting in fewer preparation errors.[9, 16] Research has shown that the EndoActivator device (EA), a sonically driven irrigant activation device, enhances irrigation effectiveness by generating robust intracanal fluid agitation in comparison to traditional needle irrigation. Rodig et al., observed a marked increase in smear layer removal when utilizing the EndoActivator instead of a canal brush and ultrasonic agitation.[17] Therefore, in our study, irrigants were activated with EA to optimize their efficacy.

In this particular research, the simulation of alveolar bone and the periodontal ligament was conducted to enhance stress distribution under occlusal pressure.[18] Silicone rubber material was chosen to replicate the periodontal ligament due to its similar elastic modulus to the viscoelastic nature of the actual ligament.[19] It was noted that using stiff materials like acrylic resin for direct embedment of roots in laboratory settings could lead to distortions in load values, potentially affecting the failure mode of specimens during mechanical testing.[20] The main objective of this study was to create a more accurate representation of in vivo conditions by simulating the periodontal ligament and utilizing an elastomeric material to cover the roots.

Fracture resistance in this research was assessed through the use of a universal testing machine. The method involved applying progressively higher vertical loads to the teeth until a fracture occurred, with the force at the point of fracture being measured in Newtons. Premolar teeth are more commonly exposed to vertical stresses rather than lateral forces during root canal treatments and occlusion. Hence, in this study, the teeth's canal orifices were vertically loaded to mimic compressive forces. A similar approach was taken in a previous study by Ayranci F. et al, where vertical loading was used to replicate occlusion forces and assess the impact of irrigants/chelating agents on root fracture resistance. This loading technique also led to a splitting stress at the access opening.[11]

The results of this study established that teeth irrigated with 9% HEBP have significantly higher fracture resistance compared to those irrigated with 17% EDTA. This finding is consistent with previous studies that have demonstrated the gentler action of HEBP on dentin.[6] By preserving more of the dentin's structural integrity, HEBP helps maintain the tooth's strength and resistance to fracture.[21]

Yamaguchi et al. (2011) compared the effects of EDTA, citric acid, and HEBP on dentin microhardness and concluded that HEBP caused the least reduction in microhardness. These findings align with the current study, highlighting the benefits of using HEBP in preserving dentin integrity.[6]

According to Kaya et al, HEBP is a selective chelator that does not cause erosion in dentin tissue. Studies have indicated that it can be used with NaOCl without compromising antibacterial properties. The selective chelating ability of HEBP minimally affects the apatite/collagen ratio and flexural strength of mineralized dentin.[22] Additionally, Osiri et al suggest that HEBP can enhance the sealer's ability to reinforce dentine by effectively removing the smear layer, thereby improving the adherence of the sealer to the root. This could potentially make endodontically treated teeth more resistant to fracture if endodontic sealers are bonded to interradicular dentin after root obturation.[23]

While the findings of this study are significant, there are several limitations to consider. The sample size limitation highlights the need for further research with larger, more diverse populations. In-vitro conditions, while providing controlled environments for testing, lack the complexity of the oral environment, such as the presence of saliva, varied microbial flora, and patient-specific factors that could influence outcomes. Additionally, the standardized irrigation protocol may not capture the nuances of clinical practice, where the application and efficacy of chelating agents can vary.

Conclusion:

This study highlights the importance of selecting appropriate chelating agents in endodontic treatment to balance effective smear layer removal with the preservation of dentin integrity. The findings suggest that 9% HEBP offers a significant advantage over 17% EDTA in terms of maintaining fracture resistance of treated teeth. Clinicians should consider these results when developing their irrigation protocols to enhance the long-term success of root canal treatments.

Clinical significance:

The research findings indicated that using 9% HEBP as a chelating agent resulted in the canals demonstrating the highest mean fracture resistance values. This suggests that it could potentially serve as a substitute for 17% EDTA as an irrigant.

List of abbreviations:

- HEBP- 1-Hydroxyethylidene-1, 1-Bisphosphonate
- EDTA- Ethylenediaminetetraacetic acid
- ANOVA- Analysis of variance
- CEJ- Cementoenamel junction

- NaOCl- Sodium hypochlorite
- CBCT- Cone beam computed tomography
- GIC- Glass ionomer cement
- RCT- Root canal treatment
- EA- endoactivator

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Figures and figure legends

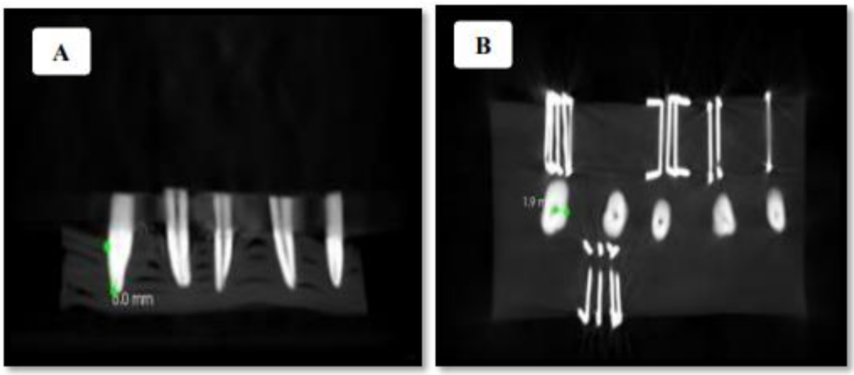


Fig. 1- Evaluation of RDT using CBCT.

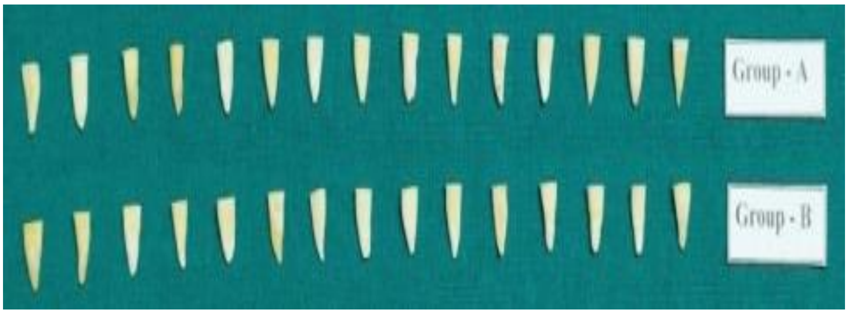


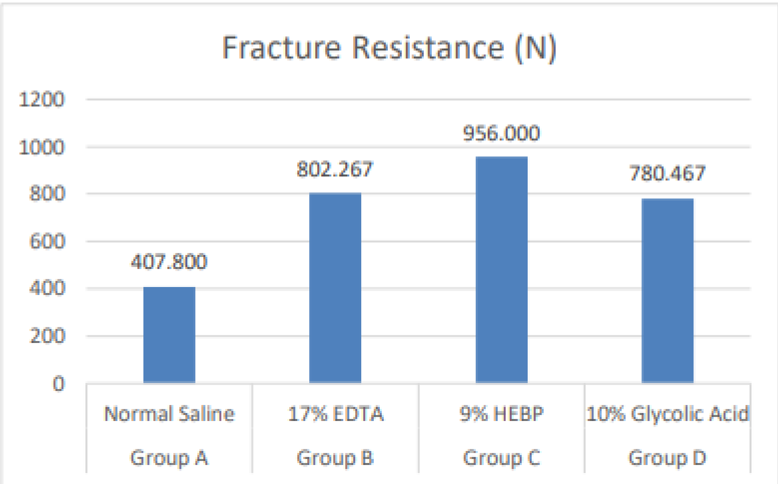
Fig. 2- Grouping of prepared specimen

Tables and table legends

GROUPS	N	Mean (N)	Standard deviation
Group A (17 % EDTA)	15	802.267	161.179
Group C (9 % HEBP)	15	956.000	206.596

Table 1- Descriptive statistics of fracture resistance values of roots irrigated with different chelating agents.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2437232.867	3	812410.956	33.964	0.000
Within Groups	1339521.067	56	23920.019		
Total	3776753.933	59			



GRAPH 1- Mean fracture resistance of roots treated with different chelating agents.