Assessment of effect of 1% phytic acid and 17% ethylenediaminetetraacetic acid on calcium ion loss of radicular dentin: An ex vivo study

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Abstract

Aim: The purpose of this study was to evaluate and compare the effectiveness of phytic acid and ethylenediamine tetraacetic acid (EDTA) in the removal of calcium ion from radicular dentin during endodontic procedure.

Materials and Methods: Twenty-five single-rooted mandibular premolar teeth were decoronated, and the roots were split longitudinally into two halves. Among obtained specimens, 45 specimens were randomly selected and divided into three groups (n = 15): Group 1 – distilled water, Group 2 – 17% EDTA, and Group 3 – 1% phytic acid. Samples in each group were immersed in the test solutions for specific time intervals, after which the same solution samples were subjected for the evaluation of amount of calcium ion release into the solution by Atomic Absorption Spectrophotometer. Data were analyzed using the one-way analysis of variance test.

Results: The use of 17% EDTA resulted in more calcium ion loss as compared to 1% phytic acid and distilled water.

Conclusion: One percent phytic acid seems to be an appropriate irrigating solution because of its less demineralizing effect as compared to 17% EDTA on radicular dentin.

Keywords: Atomic absorption spectrophotometer; calcium ion loss; ethylenediamine tetraacetic acid; phytic acid

INTRODUCTION

It is truly said, “INSTRUMENTS SHAPE, IRRIGANTS CLEAN.” Irrigation is an essential part of root canal debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone.[1]

Chelating agents are believed to aid root canal irrigation and remove the inorganic smear layer[2] as well as they play an important role by chemically softening the root canal dentin, and allowing the instrumentation of calcified, narrow canals.[3]

Root canal irrigants are capable of altering the chemical composition of human dentin and changing the calcium/phosphate (Ca/P) ratio which, in turn, affects the permeability and solubility, and their interaction with the materials used for obturation and coronal restoration as well as inhibits resistance to bacterial ingress and permitting coronal leakage.[3] Ethylenediaminetetraacetic acid (EDTA) is chemically synthesized, being overused, and is considered one of the major organic pollutants discharged in water. As EDTA is
not readily biodegradable, there have been some concerns about the leakage of this irrigant into the periapical tissue. Considering these facts, an alternative agent for smear layer removal is warranted, and the search for more biocompatible material to replace EDTA is still going on.\(^{[3,6,7]}\)

Phytic acid (inositol hexakisphosphate, IP6), a natural saturated cyclic acid, has a strong-binding affinity to important minerals, such as calcium, iron, and zinc.\(^{[8]}\) According to studies conducted by Nassar et al.\(^{[9]}\) Nikhil et al.\(^{[3]}\) and Tian Feng et al.\(^{[10]}\) phytic acid has ability to be an endodontic chelating agent as it is biocompatible, removes smear layer effectively, and has least adverse effects on microhardness of radicular dentin. To our best knowledge, there is no literature available to assess the effectiveness of phytic acid on calcium ion of radicular dentin. The present study was designed to evaluate and compare the effectiveness of 1% phytic acid and 17% EDTA on the removal of calcium ion from the radicular dentin during endodontic procedure.

**MATERIALS AND METHODS**

Twenty-five freshly extracted single-rooted human mandibular premolar teeth, free of caries, cracks, anatomic variations, immature apex, and restoration, were taken. The crowns were decoronated at cement enamel junction and longitudinally sectioned into two halves using low-speed micromotor with carborundum disc under water cooling. Among obtained specimens, 45 specimens were randomly selected and examined under the stereomicroscope to eliminate the teeth with cracks.

The specimens were then ground polished with water cooled carborundum disc. Final polishing was carried out with felt cloth and buff by using 0.05 µm size aluminum oxide powder mixed with distilled water. The external surface of specimens were isolated with nail varnish, and 45 specimens were divided into three groups (\(n = 15\)) according to irrigating solution: Group 1– distilled water (control group), Group 2– 17% EDTA (Prime Dental Products PL), and Group 3– 1% phytic acid (TCI chemical). Specimens in each group were individually immersed in a plastic jar containing 5 ml of test solution for 1 min, and then, that solution was used for calcium ion loss analysis by using the Atomic Absorption Spectrophotometer. The obtained data were analyzed statistically using the one-way analysis of variance (ANOVA) (\(P = 0.05\)) and post hoc multiple comparison analysis.

**RESULTS**

Spectrometry data were described as mean ± standard deviation, respectively, as following: Distilled water, 0.19 ± 0.04; 17% EDTA, 2.86 ± 0.3; 1% phytic acid, 1.65 ± 0.2 [Table 1 and Graph 1]. Statistical differences between the groups were determined by the one-way ANOVA (\(F = 732.47, P < 0.001\)). The post hoc multiple comparison analysis showed that 17% EDTA had more significant concentration of calcium ion in solution as compared to 1% phytic acid solution. In comparison with 1% phytic acid, there was minimal amount of calcium ion in the control group, i.e., distilled water [Table 2].

**DISCUSSION**

After enlargement of canal using any instruments (file and reamer), the dentin is covered with a smear layer which is a loosely bonded amorphous layer of organic and inorganic debris.\(^{[10,11]}\) Smear layer obliterates the dentinal tubules by reducing dentinal permeability and thus affects the penetration of obturating materials into the dentinal tubules by preventing contact between root canal wall and obturating materials.\(^{[11]}\) Therefore, irrigation has judicious role in achieving the effective root canal debridement by penetrating the mechanically inaccessible areas, flushing debris, and removing the smear layer from the root canal system.\(^{[12]}\)

The calcium ions (\(Ca^{2+}\)) present in hydroxyapatite crystals are one of the main inorganic elements of dentin. It has been reported that some chemicals used for endodontic irrigation are capable of causing alterations in the chemical composition of dentin. Any change in the Ca: P ratio may change the original proportion of organic and inorganic components, which in turn changes the microhardness, permeability, and solubility characteristics of dentin and may also adversely affect the sealing ability and adhesion of dental materials such as resin-based cements and root canal sealers to dentin.\(^{[10,4,16]}\)

EDTA reacts with the calcium ion in dentin and forms soluble calcium chelates. It has been reported that EDTA decalcifies dentin to a depth of 20–30 µm in 5 min. The decalcifying process is self-limiting because the chelator is used up. It removes smear layer resulting in better contact between canal filings and dentinal wall and better penetration of sealer into dentinal tubules. It helps in enlarging narrow, obstructed or calcified canals, bypassing fragmented instruments, lubrication, emulsification, and

### Table 1: Comparison of the calcium ion loss (ppm) (mean±standard deviation) among the three groups using the one-way analysis of variance test

| Group          | Number of samples | Mean±SD  |
|----------------|-------------------|----------|
| 1 - Distilled water | 15                  | 0.19±0.04 |
| 2 - 17% EDTA     | 15                  | 2.86±0.3  |
| 3 - 1% Phytic acid| 15                  | 1.65±0.2  |
| \(F\)           |                    | 732.47    |
| \(P\)           |                    | <0.001**  |

\(P < 0.001**\) indicates statistically highly significant results EDTA: Ethylenediamine tetraacetic acid, SD: Standard deviation
smear layer removal, but it does not have antimicrobial properties and may possess cytotoxic effects. Extrusion of EDTA through apical constriction results in an irreversible decalcification of periapical bone, and it provokes more cytotoxic effects than oxidizing potential water/sodium hypochlorite (NaOCl).[13,14]

Chemical solutions commonly used for irrigation have several undesirable characteristics such as tissue toxicity, risk of emphysema when forced beyond apex, allergic potential, disagreeable smell and taste, and biodegradability. The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives in endodontics.[15]

Herbal and natural products have been used for centuries, throughout the world in every field due to their advantages such as high anti-microbial activity, biocompatibility, anti-inflammatory, and anti-oxidant properties. In endodontics also, herbal products, for example, Curcuma longa (Turmeric), Propolis, Azadirachata indica (Neem), Aloe vera, Morinda citrifolia (noni), Triphala and Green tea, Allium sativum (garlic), Acacia nilotica (babool), German chamomile, and tea tree oil have become more popular today due to their ease of availability, cost-effectiveness, increased shelf life, low toxicity, and lack of microbial resistance when compared with conventional irrigants.[16-19]

Atomic absorption spectroscopy is a technique for determining the concentration of a particular metal element in a sample. Some studies have claimed atomic absorption spectrophotometer as one of the most effective detection systems for the determination of the trace element in dentin.[20]

In the study, calcium ion concentration was less significant in the phytic acid group ($P < 0.05$) than EDTA, which was in support with the results of the study conducted by Nikhil et al., which concluded that 17% EDTA reduced the dentin microhardness more significantly than 1% phytic acid and 0.2% chitosan. However, microhardness reduction was same for 1% phytic acid and 0.2% chitosan.[3]

The EDTA group showed significant calcium ion loss ($2.86 \pm 0.3$, $P < 0.05$) when compared with the control group. This may be attributed to its pH (acidic) value and decalcification properties. In general, the results of the present study coincides with studies conducted by González-López et al.,[21] Cobankara et al.,[11] and Spanó et al.,[20] which concluded that EDTA has greatest decalcifying capacity than other chelaters.

The results of the study conducted by Taneja et al. concluded that more calcium ion loss was seen in EDTA and showed significantly lower microhardness than the control group.[22]

Nassar et al. speculated Phytic Acid (Inositol hexakisphosphate, IP6) to have potential to replace EDTA as a root canal irrigant as it has multiple negative charges property, making it an effective chelator of multivalent cations such as calcium, magnesium, and iron. They demonstrated the ability of phytic acid (IP6) to remove the smear layer from instrumented canal and was biocompatible.[7]

An investigation on the efficacy of phytic acid carried out by Tian Feng et al. concluded that IP6 could effectively remove the smear layer from NaOCl treated dentin and did.
not cause greater variation on the chemical composition compared with EDTA.\(^9\)

The results of the present study revealed that 1% phytic acid less significantly removes calcium ion than 17% EDTA from radicular dentin. However, there was no significant calcium ion loss in the distilled water group \((P > 0.05)\).

The significant change in radicular dentin Ca/P ratio of phytic acid group can be attributed to its pH value 1.4. This acidic pH value might be responsible for the change in ratio of organic and inorganic portion of radicular dentin. This, in turn, may affect microhardness of radicular dentin. The present study was conducted on the canals which were not instrumented may be a limitation of this methodology.

**CONCLUSION**

Within the limitation of this study, it can be concluded that 1% phytic acid seems to be an appropriate irrigating solution because of its less demineralizing effect as compared to 17% EDTA on radicular dentin. Further *in vitro* and *in vivo* studies are required to evaluate the efficacy of the phytic acid to be used as endodontic irrigant clinically.

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**Conflicts of interest**

There are no conflicts of interest.

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