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**Original Article**

# EFFECT OF EDTA, ETIDRONIC ACID, PHYTIC ACID AND Er:YSGG LASER ON CALCIUM LOSS OF ROOT DENTIN USING ATOMIC ABSORPTION SPECTROPHOTOMETER. -AN *IN VITRO* STUDY

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ABSTRACT

Aim: To analyze the calcium loss of root dentin with the use of EDTA, etidronic acid, phtytic acid and Er:YSGG laser an atomic absorption spectrophotometer analysis.

Materials and Methods: Twenty samples of extracted single rooted mandibular premolar teeth were decoronated at cemento-enamel junction and were further sectioned mesio-distally into two halves. The resultant forty samples were grouped in 5 groups, GROUP A (sodium hypochlorite and EDTA), GROUP B (sodium hypochlorite and etidronic acid), GROUP C (sodium hypochlorite and phytic acid), GROUP D (sodium hypochlorite and Er:YSGG laser) and CONTROL GROUP(sodium hypochlorite and distilled water)

All the specimens were immersed in a magnetic stirrer bath containing 10 ml of the first test solution for 5 min and subsequently in second test solution. Loss of calcium in root dentin analysed by the atomic absorption spectrophotometer.

Result: Irrigation with 3% NaOCl + 9% Etidronic acid caused the maximum calcium loss from root dentin. Irrigation with 3% NaOCl + Distilled water caused minimum calcium loss from root dentin and maximum micro hardness.

## Introduction

Endodontic therapy mainly consist of biomechanical preparation, microbial control and complete obturation of the canal space.[1] During biomechanical preparation of root canals, an amorphous irregular layer known as the smear layer, that covers the instrumented walls. This smear layer consist of both organic and inorganic substance that consist of microbial debris, odontoblastic process and necrotic debris.[2] Smear layer presence increases the microflora and the inorganic toxins. It also decreases the sealing ability and increases the microbial reproduction.[3]

Irrigation believed to be the most common method to remove microorganism and bacterial products.[4] various irrigation materials are available, most commonly use irrigant is sodium hypochlorite. Sodium hypochlorite is only capable of removing organic layer. But for removal of inorganic layer, decalcifying agent such as acid or chelating agent can be used.[5]

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Fig1. laser tip

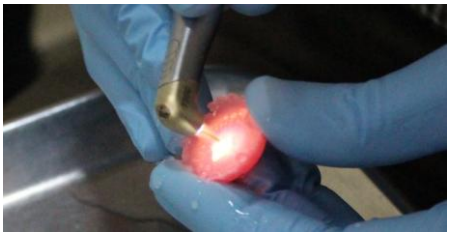


Fig 2. laser irrigation of specimen


Fig 3. Magnetic stirrer bath

Fig4. Atomic absorption spectrophotometer

Chelating agents act on the inorganic layer of smear layer and are capable of altering the chemical composition of root dentin. Various ions present on the hydroxyapatite crystal of root dentin, in which calcium ions are one of the main component.[6] Any change in the Ca:Po4 ratio it alters the microhardness, permeability, solubility characteristics of dentin as well as adhesion of dental materials and sealing ability such as resin-based cements and root canal sealers to dentin.[7]

Etidronic acid also known as hydroxyethylidene bisphosphonate (HEBP), a substance that prevents bone resorption and is used systemically in patients suffering from osteoporosis or Paget's disease. It is considered as weak chelator and fewer effect was observed on the dentin structure. When mixed with NaOCl, it shows greater chelating power and it maintains the antimicrobial property of NaOCl. [8]

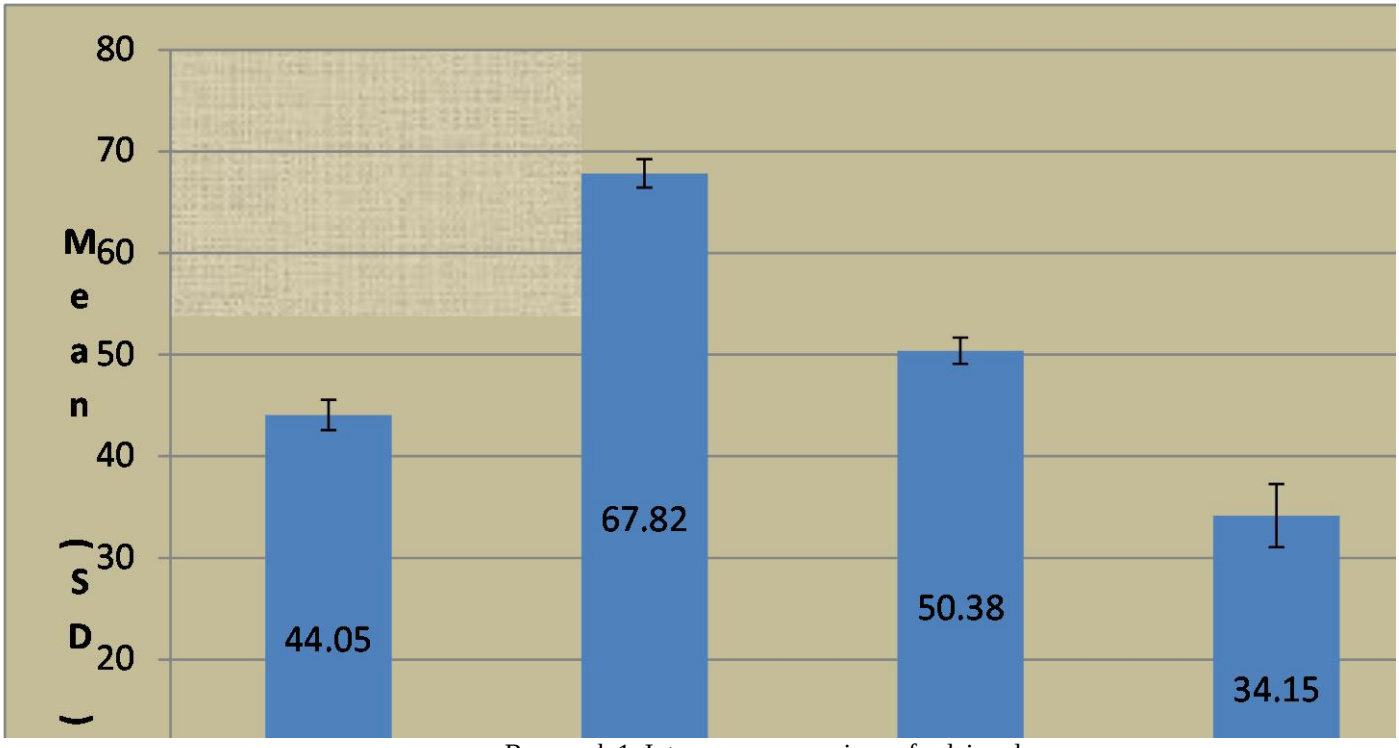
Phytic acid also known as inositol hexakisphosphate, is the major storage form of phosphorus in plant seeds and bran that contributes in a variety of cellular functions. It is also found in mammalian cells, with a concentration ranging from 10 to 100 mmol/L.[9] IP6 can be extracted with low cost from rice bran . Phytic has multiple negative charges, making it an effective chelator of multivalent cations such as calcium (Ca+2), magnesium, and iron.[10]

Erbium: Yttrium-Scandium-Gallium-Garnet (Er: YSGG) laser provides the most suitable wavelength that is 2,780 nm and approved for the cleaning and shaping of the root canal by the Food and Drug Administration (FDA).[11] The Er,:YSGG laser can remove calcified hard tissues by emitting a beam of infrared energy at 2.78 μm.[12,13,14.]

Therefore, the present study has been undertaken to evaluate the effect of different irrigation regimens on calcium loss and its effect on the micro-hardness of the root dentin.

**2.MATERIALS AND METHOD**

Twenty single rooted mandibular premolars were collected and were stored for 1 week in 10% formalin and then in normal saline until use. The premolars were decoronated at the cementoenamel junction using a high speed carbide bur under copious water irrigation and sectioned mesio-distally into two halves using a




Bar graph 1: Intergroup comparison of calcium loss

high speed diamond disc. Total fourty samples were collected and divided into 5 groups, 8 in each group.

**2.1 Preparation of irrigant.**

17% EDTA was prepared by adding 17 g of disodium salt of EDTA powder into 100 ml of distilled water.3 9% etidronate prepared by adding 9 ml of etidronate liquid into 100 ml of distilled water and 1% phytic acid is prepared by adding 1 ml of phytic acid liquid into 100ml of distilled water.[7,9,10.]

**2.2 The treatment groups were as follows:**

- Group A: 5% NaOCl for 5 min- EDTA for 5 min
- Group B: 5% NaOCl for 5 min- Etidronate for 5 min
- Group C: 5% NaOCl for 5 min-1% Phytic for 5 min
- Group D: 5% NaOCl for 5 min- Er: YSGG for 5 min.
- Group E: (Control): 5% NaOCl for 5 min-1% distilled for 5 min

All the specimens were immersed in a magnetic stirrer bath containing 10 ml of the first test solution for 5 min. The specimens were removed and rinsed thoroughly with distilled water. Then immersed into 10 ml of the second test solution of the respective group for another 5 min. For Er: YSGG laser of a 2078 nm wavelength (Biolase,India) was applied with a fiberoptic tip 300 μm in diameter, movement from the apex to the coronal region with the following parameters: Repetition rate=10 Hz, output energy=1 W, The laser was applied 5 times, and each application lasted 5 s (a total of 25 s) with 10s intervals.

Each time after irrigation of one specimen per group, the eluates were centrifuged at 820g for 10 min. The 20 ml of total eluate per specimen were collected in individual glass vials.

Once all the eluates had been collected and were analyzed for their calcium content using an atomic absorption spectrophotometer (ARM LAB,India) with an air acetylene flame. Results are expressed as ppm $Ca^{2+}$ in the eluate.

**2.3 STASTICAL ANALYSIS:**

- Comparison of the calcium loss of root dentin with the use of ethyldiaminetetraacetic acid, etidronate, phtytic acid and Er:YSGG laser using one way ANOVA test.
- p value < 0.05 was considered as significant.

**3. RESULT:**

The mean calcium loss and its standard deviation along with intergroup comparison were calculated by ANOVA test.

In this study, control group that is NaOCl + distilled water (4.30±0.6 ppm) and NaOCl + Er: YSGG (34.15±3.1 ppm) are least followed by group with phytic acid, etidronic acid. Group A shows less calcium loss (44.05±1.5ppm) than group B (67.82±1.4ppm) and group C (50.38±1.3ppm). There was a statistically significant difference between all groups. F = 1429.902 and $P < 0.001$.

**4. DISCUSSION**

Dentin is composed of inorganic components, in which calcium and phosphorus are distributed in the form of hydroxyapatite crystals. The Ca/P ratio in hydroxyapatite is approximately 1.67 and it depends on many factors such as level of mineralization, type of crystals, age of tissue and anatomical site.[15]

During biomechanical preparation of root canal, smear layer formed on the root dentin. Smear layer require the irrigant that can remove both organic and inorganic part.[16] However, reports have been indicated that the use of EDTA and NaOCl may lead to dentinal erosion of root canal walls. Further it has been reported that the surface treatment of root dentin with different agents lead to alteration in the structural and chemical composition of root dentin.[17] Atomic absorption spectroscopy used in this study to evaluate the demineralization effect of the chelators and to determine the concentration of calcium in each sample. It is a single element technique which is less cost-effective than newer multi-element, techniques such as inductively coupled plasma atomic emission spectrometry.

A study conducted by Hengameh Ashraf et al.(2014) conclude that EDTA showed significantly greater efficacy in removing smear layer followed by Er: YAG laser than etidronate.[18] A similar study conducted by Vineeta Nikhil et al (2016) showed 17% EDTA reduced the dentin microhardness more significantly than 1% phytic acid and 0.2% chitosan.[19] Hemant Kumar et al (2016) conducted a study on calcium of root dentin using eidronic acid, BioPure MTAD and SmearClear**,** it showed calcium loss is around 16.36±0.27ug/ml for etidronic acid which is similar to that of SmearClear .[20] These finding showed that microhardness depend upon the chemical composition of radicular dentin, particularly any alteration in the Ca/PO level.

In this study, irrigation with 3% NaOCl followed by distilled water as a final rinse eluted minimum amount

of calcium from the dentin (4.3 ppm mean) and when compared with other groups, the difference was statistically significant. Higher calcium loss is seen with etidronate (67.82 ppm). Whereas Er:Ysgg laser shows less calcium loss (34.15ppm) than EDTA and Phytic acid acid. Er:Ysgg laser.

This finding is in agreement with a study conducted by Lottanti *et al.* (2009) where NaOCl and distilled water hardly eluted any Ca in the control group, no chelating agent was used but still some calcium loss was seen as a result of its mechanical flushing action on smear layer formed on root dentin. Since chelating agents cause demineralization of dentin, resulting in its softening.[21] Hemant Kumar et al (2016) concluded that the chelating efficiency of 18% HEBP was found better than 9% HEBP because of higher concentration. A less aggressive calcium complexing agent such as 7–10% HEBP could be administered during the whole course of root canal preparation to prevent erosive dentinal changes.[20]

In our study we find that more Ca loss was seen in groups having EDTA, PHYTIC ACID AND Er:YSGG LASER.

With maximum calcium loss with etidronate (67.82 ppm), may results in maximum alteration in $CA/PO_4$ and maximum loss of microhardness. Where as Er:ysgg laser shows less calcium loss (34.15ppm) than EDTA and Phytic acid.

**5. Conclusion:**

- Irrigation with 3% NaOCl + 9% ETIDRONATE caused the maximum calcium loss from root dentin. Irrigation with 3% NaOCl + Distilled water caused minimum calcium loss from root dentin and maximum microhardness.
- With increase in calcium loss from root dentin in turn changes the microhardness, permeability, and solubility characteristics of dentin.

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