Effectiveness of Various Endodontic Irrigants on the Microhardness of the Root Canal Dentin-An *In vitro* Study

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Abstract

Background: Different Irrigating solutions used for root canal treatment have different physical and chemical properties, which will render teeth fracture. Therefore proper selection of irrigant is needed to have maximum benefits. **Aim:** The aim of this study was to evaluate the effect of guava leaf extract as endodontic irrigant on microhardness of root canal dentin. **Materials & Methods:** Freshly extracted single rooted teeth were taken for the study. The crown portion of the tooth was sectioned at the level of CEJ and the root portion is sectioned longitudinally. These sections will be embedded in resin mould and divided into 3 groups as guava leaf extract, sodium hypochlorite and 17% EDTA with 10 samples per group for 20 minutes. Then the teeth were subjected to vickers hardness test. **Results:** The results were expressed as Mean ± SD (n=10). The *p<0.05 statistically significant as compared with the pretest group. p<0.01 statistically significant as compared with 3% NaOCl and EDTA 17% group. **Conclusion:** The study results showed better microhardness in guava leaf extract followed by 17% EDTA and 3% sodium hypochlorite. Guava leaf extract can be used as an alternative irrigant in endodontics.

Keywords:

Microhardness; Irrigant; Natural irrigant; Endodontics; Guava leaf extract

Introduction

Micro organisms like bacteria and their products play an essential role in the pathogenesis of pulpo-periapical diseases.

A long-standing endodontic infection allows bacteria to propagate to the entire root canal system, including ramifications, isthmuses, apical deltas and dentinal tubules. [1–3]

In contemporary endodontics, chemomechanical preparation associated with antiseptic medication has been recommended for infection control.

Despite this, residual microorganisms may persist in root canals. [4]

Anatomical complexities and microbiological factors often pose serious threats to adequate root canal disinfection.

It is a prerequisite to use endodontic irrigants in addition to mechanical preparation to ensure the success of root canal treatment. [5]

Root canal instrumentation comprises the combined action of endodontic instruments, chelating agents and irrigating solutions which is aimed at the elimination of pre-existing organic and inorganic debris resulting from the operative procedures as well as the reduction of the microbial content and it's by products. [6]

Irrigating solutions used during endodontic treatment may lead to alterations in the chemical structure which may in turn affect the mechanical properties of dentin.

These irrigants used for removal of the smear layer may act similarly on the smear layer as well as the root dentin.

Irrigating solutions also help to reach the complicated structures in root canal like accessory and lateral canals.

It consequently leads to the exposure of collagen and eventually causes decrease in dentin microhardness.

Pashley et al., suggested that an inverse relation exists between the dentin micro-hardness and density of the dentinal tubules.

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Reduced micro-hardness may lead to reduction in modulus of elasticity and flexural strength of dentin. [7] Hence the determination of micro-hardness provides an arbitrary assessment of the change in any mineral content of dental hard tissues.

Sodium Hypochlorite (NaOCl) has been the most widely used root canal irrigating solution for several decades.

It is considered to be the gold standard due to its properties of tissue dissolution and antimicrobial activity making it the irrigating solution of choice for treatment of teeth with pulp necrosis.

This holds true that sodium hypochlorite has various undesirable characteristics such as tissue toxicity, risk of emphysema when overfilling, allergic potential, a disagreeable smell and taste.

Moreover, it might not completely cleanse the surfaces of the root canal walls. [8]

Chlorhexidine (CHX) is effective against both gram-positive and gram-negative microorganisms since it is proven broad-spectrum antimicrobial agent and due its substantive nature.

It is the most potent chemotherapeutic agent against many microbes. At low concentration, it is bacteriostatic and at high concentration bactericidal. Side effects of chlorhexidine produces staining of teeth, altered taste, and development of microbial resistance. [9]

Various irrigation activation systems such as sonic and ultrasonic systems have also been used to increase the efficacy of sodium hypochlorite in cleaning the canal system along with lateral canals and provide improved penetration of hypochlorite into the biofilms to eradicate the endodontic microbes. [10]

The use of herbal plant extracts for the eradication of microbes has been the topic of interest due to the drawbacks of sodium hypochlorite and chlorhexidine.

Herbal extracts such as Morindacitrifolia, Green tea, *Triphala*, *Azadirachta Indica* etc. have been used as irrigants in various studies. These studies have proven that herbal plant extracts eliminate microbes causing dental pathologies, thus proving its efficacy as an antimicrobial for oral infections. [11,12] We have numerous highly cited publications on well designed clinical trials and lab studies. [13–27]

Psidium guajava is a phytotherapic plant commonly known as Guava. It belongs to the Family Myrtaceae species. [28,29] The leaves of the plant *P. guajava* Linn are reported to possess antioxidant, hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anti cough, antidiabetic, anti inflammatory and antinociceptive activities. [30–34]

With the increasing popularity of traditional and holistic/alternative medicines due to their natural origin, easy availability, efficacy, safety and fewer side effects. Previously our team has a rich experience in working on various research projects across multiple disciplines. [35–49] Now the growing trend in this area motivated us to pursue this project. The aim of this study was to evaluate the effect of *Psidium guajava* (guava) leaf extract as endodontic irrigant on microhardness of root canal dentin.

Materials and Methods

Specimen preparation

Freshly extracted 30 single rooted teeth were selected for the study and stored in 0.1% thymol solution until its use

. Non carious, non fractured, non restored, single rooted teeth were included in the study and the carious, fractured and multi rooted teeth were excluded from the study.

The selected samples were decoronated at the level of Cemento-Enamel Junction (CEJ) with the help of diamond disc in the presence of water coolant.

Then the samples were divided into 3 groups, Group 1-17% Ethylene Diamine Tetraacetic Acid (EDTA), Group 2-3% Sodium Hypochlorite (NaOCl) and Group 3-20% Guava leaf extract, containing 10 samples in each group.

Then the tooth were sectioned longitudinally and mounted on acrylic blocks [Figure 1].

(a) (b)

Figure 1: (a) Samples were decoronated at CEJ, (b) Mounted on acrylic blocks.

Treatment groups

These acrylic blocks with the sectioned tooth were subjected to Vickers hardness test and recorded as pre test evaluation (Baseline). Then the specimens in Group 1 (n=10) were immersed in EDTA 17%.

The specimens in Group 2 (n=10) were immersed in 3% NaOCl. The specimens in Group 3 (n=10) were immersed in 20% Guava Leaf Extract (GLE) for 30 mins. The solution was kept at room temperature.

After that each tooth was rinsed with distilled water and dried.

Surface microhardness measurement

The surface microhardness which was measured as a pre test evaluation is the baseline using Vickers microhardness tester. After the treatment the vickers hardness test was done for all the groups.The microhardness tester is equipped with a 400X magnification lens that enables a clear view of the indentation created by the microprobe.

The created indentation has 2 diameters of X and Y that are precisely measured by the device and reported as d1 and d2. The microhardness is calculated using the mean d1 and d2 and the formula below:D: The microhardness number is inversely correlated with the d value.

The greater the d1, d2 and consequently the total d value, the higher the penetration of indenter into the surface and the lower the microhardness number of the object and vice versa.

In order to confirm the accuracy of the obtained microhardness value, each specimen was tested 3 times and the mean of all values was reported as the microhardness number. During the experiment, the solutions had a temperature equal to the room temperature (approximately 30°C).

After immersion for 5 min, the specimens were rinsed with saline solution, dried and their surface microhardness was measured again.

Statistical analysis

Surface microhardness was measured with Vickers hardness test and the data were analyzed using one-way ANOVA with Dunnett's T3 test. $p<0.05$ was considered statistically significant.

Results and Observations

The results were expressed as Mean ± SD (n=10). The *$p<0.05$ statistically significant as compared with the pretest group. $p<0.01$ statistically significant as compared with 3% NaOCl and EDTA 17% group [Figure 2, Table 1].

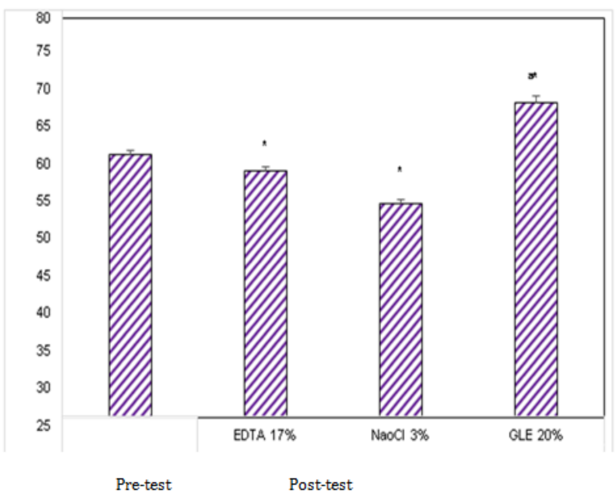

Figure 2: This figure shows the micro hardness testing results of treatment groups.

Table 1: Table shows the vickers hardness test of the treatment groups.

Groups	Sample size (n)	Vickers Hardness test (VHN) mean		Vickers Hardness test (VHN) Standard Deviation (SD)		P-value
		Pre test	Post test	Pre test	Post test	
Group 1 (17% EDTA)	10	53.59	49.42	2.40277	1.57970	<0.05
Group 2 (3% NaOCl)	10	51.462	42.826	5.05306	1.71007	<0.001
Group 3 (20% GLE)	10	52.878	63.176	1.66061	3.93666	<0.001

Discussion

The present in-vitro study evaluated the microhardness of *Psidium guajava* leaf extract on the root canal dentin as an irrigant. The results showed that there is a significant difference between group 1 (17% EDTA) and group 2 (3% NaOCl) and group 3 (20% GLE). The guava leaf extract (GLE 20%) showed better results with microhardness to root canal dentin. This guava leaf extract group did not reduce or affect the microhardness of the root canal dentin.

The main goal of root canal treatment is to clean the canal by considering biological, chemical, and mechanical objectives. [50,51]

After mechanical debridement, biofilm may remain undisturbed in the anatomically challenging areas such as fins, lateral or furcal canals, apical deltas, webs, and isthmus. Effective disinfection in endodontics is only achieved by augmenting mechanical preparation with antimicrobial irrigants.

Those irrigants which are used in endodontics to remove the root canal bacteria will have some effect on the root canal dentin, which in turn affect the sealing ability with the obturating material, fracture resistance. [52,53]

Hence to use an agent as endodontic irrigant clinically it should not have the cytotoxic effect on the periapical tissues, should help in healing of periapical tissues and lesions, should possess antimicrobial properties, should be able to remove the smear layer and it should not alter the physical properties of tooth structures like root canal dentin. [54–56]

Psidium guajava (Guava) is considered as a poor man's apple rich in phytochemicals with medicinal value and hence it is highly consumed. Gas Chromatography–Mass Spectroscopy

(GC–MS) analysis of guava leaf extract revealed the presence of various bioactive compounds with antimicrobial, antioxidant, anticancer, and antitumor properties. [57] The flavonoids such as mosin glycosides, quercetin, and quercetin glycosides may contribute to antibacterial action of guava leaf extracts. [58] The resistance to bacterial attacks suggested being as a result of the polygalacturonase inhibitory proteins in the plant cell walls of guava. The aqueous extracts of guava leaf can cause a marked reduction in the adhesion of the early organisms of plaque biofilm formation. [59]

The phytochemicals present in guava leaf extract are Caryophyllene, α-copaene, cis-muurola-3,5-diene, humulene, cyclosativene, bicycle (5.3.0) decane, 2-methylene-5-(1-methylvinyl)-8-methyl,1H-benzocycloheptene,2,4a, 5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-,(4aS-cis), 1H-cyclopropa[a]naphthalene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl,[1aR-(1aa,7a,7aa,7ba)], naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl), (1S-cis), α-cadinol, α-bisabolol etc. are some of the sesquiterpenes identified in the guava leaf extract. [60,61] These compounds are well known for their antimicrobial, anti-inflammatory, antioxidant, antiproliferative, anticancer, antitumors and anaesthetic properties. [62–65] Our institution is passionate about high quality evidence based research and has excelled in various fields. [66–72] We hope this study adds to this rich legacy.

In this study, the guava leaf extract (GLE 20%) has been used as an endodontic irrigant on the root canal dentin. The results showed that the guava leaf extract (GLE 20%) did not affect the microhardness of the root dentin, followed by 3% NaOCl and 17% EDTA. There is a significant difference between guava leaf extract 20% and EDTA 17%. As it does not affect the microhardness of the root canal dentin and few studies have shown that it has good antibacterial effects, this guava leaf extract can be considered as one of the irrigating solutions in future.

Conclusion

Within the limitations of the study, results showed better microhardness in guava leaf extract followed by 17% EDTA and 3% sodium hypochlorite. It is considered to possess all other properties to be an endodontic irrigant like cytotoxicity, antibacterial effects etc. Hence, in future guava leaf extract can be used as an alternative irrigant in endodontics.

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