ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
June 2021 • Vol. XXIV

The Effect of Two Natural Irrigations on Canal Dentine Microhardness. (In-Vitro Study).

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Abstract

Purpose: Successful endodontic therapy is based mainly upon effective mechanical instrumentation and disinfection of root canals. This study compares the antibacterial effect of Erbium, chromium: yttrium-scandium-gallium-garnet (Er, Cr:YSGG) laser 2780nm wavelength, diode laser 940nm wavelength and sodium hypochlorite (NaOCl) 5.25% solution on *Enterococcus faecalis* (*E. faecalis*) biofilm.

Materials and methods: A total of 50 extracted human permanent maxillary central incisor teeth were prepared to size X4 Protaper Next and contaminated with *E. Faecalis* for biofilm formation. After ten days of incubation and based on the method of disinfection of the root canals, the specimens were randomly grouped into; group A (n=15), which was irradiated by ER, Cr: YSGG laser 2780nm wavelength; group B (n=15), which was irradiated with a diode laser 940 nm wavelength; group C (n=15), which was rinsed with 5.25% NaOCl solution. Three teeth were used to confirm the biofilms formation by scanning electron microscope (SEM) and two teeth served as negative control. Intracanal bacteria sampling was performed under complete aseptic conditions before and after disinfection. The specimens were cultured to enumerate the colony forming units (CFUs) count.

Results: Although group C (5.25% NaOCl solution) showed a more disinfection than both laser systems, there was no significant difference between all groups (P> 0.05).

Conclusion: Both ER, Cr: YSGG laser 2780nm wavelength and diode laser 940nm wavelength have nearly similar antibacterial efficacy on *E. faecalis* compared with NaOCl 5.25% solution. The use of laser for root canal disinfection may be advantageous considering several unfavorable actions of NaOCl.

Key words: Laser, *Enterococcus faecalis*, Root canal, Sodium hypochlorite.

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Introduction

Endodontists are facing a lot of challenges such as anatomical complexities of the morphology of pulp space such as delta, accessory canals, isthmus and ramifications and microbiological factors mainly biofilms to perform proper disinfection.(1) Root canal treatment is a chemomechanical procedure for proper disinfection, so mechanical instrumentation must be complemented by chemical irrigation. Most of the irrigants used in the root canal treatment might have an influence on the biofilm microbes but no one has all ideal requirements of the root canal irrigant such as antimicrobial action, debris flushing out, smear layer removal, dissolving vital tissues as well as necrotic ones, lubrication action, being nonirritating to the periapical tissues, has no adverse effect on the physical properties of dentin, does not interact with the sealing ability of either the root canal sealer or the obturating material, be easy to use and economic, prevents the formation of smear layer and be non-toxic to periodontal tissues. (2)

The most common irrigant is sodium hypochlorite (NaOCl) because it has potent antimicrobial property as well as its excellent dissolving of non-vital tissue.(3) Senia et al. reported that NaOCl has antibacterial activity and dissolving capacity of necrotic tissue and vital one, bacterial colonies (biofilm) and collagen matrixof dentin in a fast manner.(4) There are many disadvantages of NaOCl such as staining, allergic reaction, toxicity, bad taste, corrosion to metal instruments (5) and deficient smear layer removal because of it has no effect on inorganic component. NaOCl solution of concentration 5.25% when used for irrigation, it has exhibited excellent antibacterial property, however it has reflected high cytotoxicity for periapical tissues when it passed beyond the apex. (6) NaOCl has some drawbacks such as bad taste and trigger allergic responses in some cases. In addition, it is harmful for mucous membrane of oral cavity. The follicles of unerupted or erupting permanent tooth can be destroyed by NaOCl. It is considered as corrosive substance for metal instruments.(7) Radcliffe et al. confirmed that *Enterococcus Faecalis* exhibited high resistance to sodium hypochlorite.(8) Consequently, an effective, harmless potent alternative irrigant would be preferred. (9)

Today herbal medicine is an alternative therapy which spreads throughout the world due to better efficacy, bio compatibility, economics and easily availability.[10] And a lot of herbal products are gaining popularity in dentistry because they have potent anibacterial and anti-inflammatory actions, some of these natural extracts are aloe vera and propolis.

Aloe Vera (Aloe Barbadensis Miller) has antimicrobial action against resistant microorganisms found in root canal system. Aloe vera includes amino acids, enzymes, vitamins, saponins, lignin, salicylic acids, minerals, and sugars also, has bacteriostatic effect due to presence of latex compound in aloe vera. Anthraquinones have antibacterial effect which present in aloe vera leaf extracts. [11]

Propolis is known as Bee Glue. It is natural product obtained from the honey bees. It has many therapeutic actions. It is considered as antioxidant, antibacterial, antifungal, antiviral, antitumor, and anti-inflammatory agent, so it has been used in medicine. The flavonoids which are active constituents act as anti-inflammatory agents.[12]

The exposure of coronal and radicular dentin to chemical solutions during irrigation might affect the dentin microhardness. The effectiveness of these natural extracts on the root canal dentine microhardness is not cleared and needs further investigations.

Materials and methods:

A) Samples selection and preparation:

Thirty extracted human single rooted permanent premolars were collected in this study from the oral surgery department of Ain Shams University. Teeth were inspected to exclude any obvious fracture and cracks and radiographed to exclude any resorption or calcification. then immersed in (5.25%) NaOCl solution to remove any deposits, while hard deposits were removed by ultrasonic scalar. For a subsequent step, to facilitate longitudinal splitting of roots into buccal and lingual halves, all roots were grooved on the external mesial and distal surface with a diamond disc (Komet, USA) avoiding penetration of the root canals. We stored all teeth in saline until use. All teeth were decoronated at level 16 mm from the apex to obtain standardized root length of teeth, using high speed diamond disc with water coolant. According

which irrigant used, the samples were divided to three equal groups (n = 10); Group I (90% Aloe vera), Group II (4% propolis) and Group III (2.6% NaOCl). The root was divided longitudinally into two halves using a small mallet to strike on #15 surgical blade (Heny Schein, Port Washington, NY) placed in the groove. Both halves of each sample were embedded in autopolymerized acrylic resin (Acrostone, Madenet El Salam, Egypt). One half of each sample was considered as control for its other experimental half. Dentin surface was ground to be smooth with finer polishing papers (600, 800 and 1200-grit polishing papers) and distilled water.

B) Irrigant preparation:
Preparation of 90% Aloe vera

We brought leaves of Aloe Vera plant and washed them with water, finally we used 70% ethyl alcohol to disinfect the leaves to be ready to extract the gel from inside. The leaves were opened, and the pulp was collected and homogenized. We used 10 milliliters of distilled water to dissolve 90 grams of the gel to obtain 90% Aloe Vera solution ***Sahebi et al.*** (13)

Preparation of 4% propolis: We used the solid powder of propolis 4gm (Imtenan, Cairo, Egypt) and liquid of Dimethyl Sulfoxide 100ml (Tedia company, Inc, Fairfield, USA). We mixed the powder and liquid well to obtain a concentration of 4%. The mixture was poured into a sterile flask and incubated for 24 hours to insure the dissolution of mixture. Finally filtration was done several times using filter papers to eliminate any remnants or undissolved particles.(14)

C) Irrigant application: On the exposed surface of the experimental half, we built a basin-like model of pink wax following root outline to hold the irrigant during the sample treatment. Using a plastic syringe 2 ml of each irrigant for 5 minutes was applied according to each group; Group I (90% Aloe vera), Group II (4% propolis) and Group III (2.6% NaOCl). Finally, we washed the samples with tap water to be ready for microhardness evaluation.

D) Evaluation of canal dentine microhardness: Root dentin microhardness was measured with Vicker's hardness tester. We measured the microhardness for each third of each sample. The indentation was made almost 0.5 mm from the space of the canal using a Vicker's diamond indenter by using a 200-g load for 15 seconds for each measurement. We upturned the measurements into a

value called Vicker's Hardness numbers (VHN) through the equation:

HV=1.854 P/d2.

HV= Vicker's Hardness (Kgf/mm2)

P= Load (Kgf)

d= Average length of the diagonal (mm)

Finally for each sample, we calculated the decrease in microhardness as the percentage change of dentine microhardness[15] as the flowing:

V1-V2/V1×10

Where, V1 = VHN for control half

V2 = VHN for corresponding treated half

Statistical analysis:

We collected the data and tabulated for statistical analysis. We used IBM SPSS computer software Statistics Version 26 for Windows. We used One-way ANOVA test for intergroup comparisons which was followed by Tukey's post hoc test, while intragroup comparison done by repeated measures ANOVA test followed by bonferroni post hoc test. We set the significance level as P ≤0.05.

Results

Post hoc pairwise comparisons showed microhardness percentage change (%) of samples with different irrigation materials to be significantly different from each other (p<0.001). **(Table 1).** Regarding to canal level in the present study, the coronal level was significantly higher than middle and apical levels (p<0.001) as showed by **(Table 2).**

Table (1): Mean ± standard deviation (SD) of microhardness percentage change (%) in different irrigant groups

Root section	Microhardness percentage change (%) (mean±SD)			p-value
	Aloe vera	Propolis	Sodium hypochlorite	
Coronal	-2.25±0.48^{C}	24.13±2.55^{A}	10.40±0.29^{B}	<0.001*
Middle	-3.41±0.54^{C}	20.09±3.14^{A}	8.42±0.46^{B}	<0.001*
Apical	-4.35±0.49^{C}	19.94±3.99^{A}	8.75±0.48^{B}	<0.001*
Overall	-3.40±1.00^{C}	19.08±4.70^{A}	8.45±0.55^{B}	<0.001*

*Different superscript letters exhibited a statistically significant difference in same horizontal row *; significant (p ≤ 0.05).*

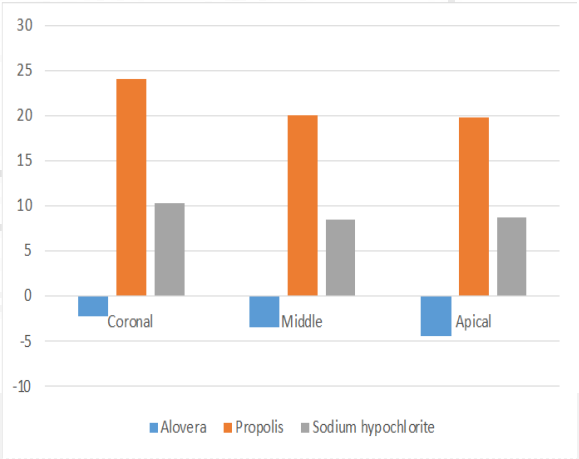


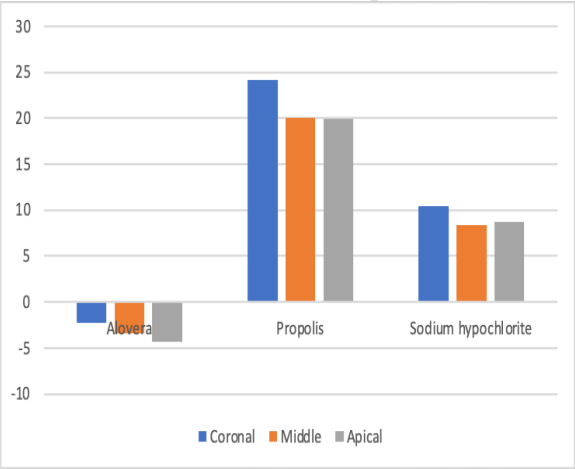
Figure (1): Bar chart showing average microhardness percentage change (%) for different irrigants at different thirds

Table (2): Mean ± standard deviation (SD) of microhardness percentage change (%) in different irrigation materials at the same third

Irrigation material	Microhardness percentage change (%) (mean±SD)			p-value
	Coronal	Middle	Apical	
Alovera	-2.25±0.48^{A}	-3.41±0.54^{B}	-4.35±0.49^{C}	<0.001*
Propolis	24.13±2.55^{A}	20.09±3.14^{B}	19.94±3.99^{B}	<0.001*
Sodium hypochlorite	10.40±0.29^{A}	8.42±0.46^{B}	8.75±0.48^{B}	<0.001*

*Different superscript letters exhibited a statistically significant difference in same horizontal row *; significant ($p \leq 0.05$)*

Figure (2): Bar chart showing average microhardness percentage change (%) for different irrigation groups



Discussion:

Success in endodontic treatment depends on chemomechanical procedure which is cleaning of the canals by mechanical means such as instruments and potent chemical solutions (irrigants). [16] The use of root canal irrigation can contribute to structural changes on the surface of root dentin, which may alter its physical features.[5,17] Meanwhile, sealing and adhesion of the sealers can be affected by microhardness changes of dentin of the root canal.

Among different types of irrigating solutions that have been used in endodontic treatment and to date, NaOCl is ranked the first irrigant used worldwide due to its superior antimicrobial [8] and an extremely effective tissue dissolving properties, it dissolve organic and necrotic tissue very efficiently. [18] Many natural elements have been studied as an irrigant in order to avoid the serious drawbacks of the commonly used root canal irrigants (Murray et al., 2008), [19] as the use of NaOCl does not come without reservations due to the high incidence of NaOCl accidents, toxicity, hypersenstivity at some patients when accidentally injected beyond the root apex, followed by extreme pain, edema, and hematoma (Virtej et al. 2007)[20] and the great resistance of *E. faecalis* to hypochlorite (Radcliffe et al., 2004).[8]

Endodontists have used herbal substances since early times to avoid the cytotoxic effect of many used

irrigants and to be able to eliminate bacteria which specifically found inside dentinal tubules of root canal system. New medicine has focused to use the extract of natural plants.[21].

Aloe Vera (Aloe Barbadensis Miller) has high biocombatability. It is powerful anti-inflammatory antibacterial, and antifungal agent, so many studies have been done to examine its application as an irrigant and medicament during root canal treatment. [22]

Propolis has a yellowish brown tint. It is natural antibiotic resinous material which honey bees collect from tree buds. It consistes of different chemical compounds mainly phenolics and flavonoids. Flavonoid is chemical compound which presnt in may plants. Flavonoids are potant antibacterial and antifungal compounds, moreover they are antiviral. They expressed high anti-inflammatory and antioxidants capability (Fausto Rodrigo Victorino et al, 2007). [23]

Laboratory studies must be done before using new irrigation to investigate the benefits and consequences. Consequently, the our study aimed to investegate the result in terms of the percentage of change in the micro-hardness of dentin wall of root canal using different three irrigants (90% Aloe Vera solution and 4% propolis solution &, 2.6% NaOCl). Teeth were decoronated to a length 16 mm to standardize all samples. On the mesial and distal surfaces we cut longitudinal grooves to facilitate root splitting without griding in order to avoid introduction of debris inside the canal. [24] Standardisation of irrigant volume was considered to attain similar circumstances for all the samples and to avoid any bias in the results.[25]

In the present study, the longitudinal sectioning of the roots was preferred instead of transvers sections as Cruz-Filho et al observed that it can display exact representations of clinical situations. The Vickers tester was used in the present study as it is less sensitive to surface conditions.[26] In the present study, it was found that: 90% Aloe vera has increasd the dentine microhadness, this result was in agreement with **Silva et al.** [27] who concluded that aloe vera increased superficial microhardness. Aloe vera gel is formed of diffrent chemical structures. It is mainly formed of water with percentage of 98-99% and lesser amount of active components 1-2% such as, anthraquinones, flavonoids, naphthoquinones, , polysaccharides,

vitamins, essential amino acids mainly arginine, uronic acid, salicylic acid, and galacturonic acid; glucose mannose, fructose, and other hydrolysable sugars; enzymes as catalase, oxidase, and amylase and different menirals; calcium, magnesium, sodium and potassium. So, deposition of the calcium on the surface of dentin might be responsible for remineralizing capability of 90% Aloe vera solution as reported by **Silva et al.** [27] Hardness property of dentin can be affected by changes in mineralization.[28] so the aloe vera increases the surface microhardness of dentin wall of the root.

Our results from this study revealed that 4% Propolis and 2.6% NaOCl significantly decreased the microhardness of dentin wall of root canal however the effect of 4% propolis was more pronounced, this result was in agreement with **Elgendy, *A. A***. [14]

The causative factor in the significant reduction in dentin microhardness caused by 4% propolis might be due to flavonoids and esters of phenolic acids resent in propolis . Phenolics are weak acids have chelating action . [29] While, the dissolving effect for dentin collagen, phosphate and magnesium ions is the causative factor in the significant reduction of dentin microhardness after use of 2.6% NaOCl.[17] Moreover, it was proved by **Dogan, 2001** that NaOCl treatment altered significantly the ratio of Ca/P of the root dentin. [30] The hardness property of dentin can be affected by changes in mineralization. [28] So the reduction in dentin microhardness might be attributed to the changes in the mineral content of root dentin after NaOCl irrigation

Regarding to canal level in the present study, the coronal level was significantly higher than middle and apical levels ($p<0.001$). This finding was in agreement with **(Nikhil et al. and Massoud et al.)** [31,32] This might be due to histological structure of dentin and the dentin nature at apical part as Carrigan et al. [33] showed that there was decrease in tubule density of dentin from coronal part to apical part. Pashley et al. [34] revealed that there was an inverse correlation between tubular density and dentine microhardness.

Conclusion

Under the condition of the present study, it can be concluded that, 90% Aloe vera as root canal irrigant improved dentin microhardness, however both 4% propolis and 2.6%

NaOCl decreased microhardness of root canal dentin.

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