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AN *IN*-VITRO COMPARATIVE SEM STUDY OF APPLE CIDER VINEGAR, PALM VINEGAR, POMEGRANATE VINEGAR AND GRAPE VINEGAR ON THE SMEAR LAYER REMOVAL OF ROOT CANALS

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ABSTRACT

The aim of this in-vitro comparative study was to evaluate and compare in-vitro, by scanning electron microscopy (SEM) the removal of smear layer at the coronal, middle and apical third of root canals irrigated with four natural vinegars which are Apple Cider Vinegar, Palm Vinegar, Pomegranate Vinegar and Grape Vinegar. The objective was to investigate which of the four types of natural vinegars (Apple Cider Vinegar, Palm Vinegar, Pomegranate Vinegar and Grape Vinegar) could be used as the better alternative to a chemical (Sodium hypochlorite), in the removal of smear layer, when used as a root canal irrigant. Fifty human maxillary central incisors were instrumented and the final irrigation was performed with apple cider vinegar, palm vinegar, pomegranate vinegar, grape vinegar and 2.5% sodium hypochlorite (control). Smear layer removal was assessed in the cervical, middle, and apical thirds of each specimen under SEM. There was statistically significant difference ($P < 0.001$) between apple cider vinegar and theother solutions with regard to smear layer removal. The highest amount of smear layer removal was obtained with apple cider vinegar followed by palm vinegar, grape vinegar and pomegranate vinegar.

Key words: Apple Cider Vinegar, Smear layer removal, Sodium hypochlorite, Scanning Electron.

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INTRODUCTION

Dentin debris and smear layer (SL) are created on the root canal walls as a consequence of endodontic instrumentation. According to the American Association of Endodontists, SL is defined as a surface film of debris retained on dentin or other surfaces after instrumentation with either rotary or endodontic files, consisting of remnants of vital or necrotic pulp tissue, dentin particles, retained irrigant, and bacterial components. It results in obliteration of dentinal tubules making it difficult to eliminate microorganisms and compromises the filling of the root canal systems(Torabinejad *et al.,* 2004).

No irrigating solution used in endodontic treatment is capable of acting on the organic and inorganic elements of the smear layer simultaneously. Sodium hypochlorite (NaOCl), in concentrations of 0.5% to 5.25%, is the main endodontic irrigant, but when used

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alone is ineffective in removing the entire smear layer. (Mc Comb and Smith, 1975; Mader and Baumgartner, 1984; Torabinejad *et al.,* 2002) Chelating agents are used in endodontics to aid in root canal irrigation and to remove the inorganic smear layer. The ethylenediaminetetraacetic acid (EDTA) at a neutral pH has been recommended since 1957 and it is the one most frequently employed for the removal of the smear layer (Torabinejad *et al.,* 2003).

The cleaning action of irrigants is reduced toward the apex and is less efficient in the apical region of the root canal (O'Connell *et al.,,* 2000; Khedmat and Shokouhinejad, 2008) This could be attributed to the narrow dimensions of the apical third, which can prevent the effective distribution of irrigants, resulting in limited contact between the canal walls and the solutions.(Ciucchi *et al.,* 1989)

Sodium hypochlorite (NaOCl) is one of the most widely used endodontic irrigants for the chemomechanical preparation of root canals because of its excellent antimicrobial action and capacity of dissolving organic materials (Marending *et al.,* 2007), which increase directly with the increase of the concentration. However, the optimal organic tissue-dissolving property of NaOCl is non-selective, which means that, especially at high concentrations, this chemical agent may dissolve both vital and necrotic pulp remnants indistinguishably and have high toxicity to the periapical tissues in case of inadvertent extrusion through the apical foramen to the periradicular space (Kuruvilla and Kamath, 1998). Another disadvantage of NaOCl is that it decreases the mechanical resistance of dentin (Marending *et al.,* 2007) by causing deterioration of collagen and proteoglycans. There are also reports of accidents and allergic reactions to the use of NaOCl during root canal therapy (Pelka M and Petschelt A, 2008; Pontes *et al.,* 2008). Therefore, research has been done to find an irrigating solution that may have better biocompatibility than NaOCl while maintaining its properties of tissue solving capacity and high bactericidal action. Vinegar has been indicated as an antiseptic agent due to its medicinal properties and has been used for the treatment of infected wounds. Distilled white vinegar and wine vinegar are composed mainly of acetic acid, whereas apple vinegar is composed mainly of malic acid, which has therapeutic properties (Caligiani *et al.,* 2007). More recently, the use of apple vinegar as an auxiliary solution in the chemomechanical preparation of root canals has also been investigated and deserves attention due to the promising results obtained when compared to traditional endodontic irrigants, such as NaOCl and EDTA (Costa *et al.,* 2009).

Other substances have also been suggested to remove the smear layer, such as citric acid and apple vinegar (Canderio *et al.,* 2001) Apple vinegar is composed of 5% acetic acid and 0.35% malic acid (Caligiani *et al.,* 2007) It has good cost-effectiveness and is a biocompatible substance. Its antimicrobial potential has already been demonstrated, (Estrala *et al.,* 2004) but little published data is available regarding its cleaning ability. Apple vinegar associates a good capacity to remove smear layer from the dentinal tubule entrances (Estrala *et al.,* 2007; Zandim *et al.,* 2004) with bactericidal action against microorganisms that are frequently associated with endodontic infections, such as *Staphylococcus aureus* and *Enterococcus faecalis* (Estrala *et al.,* 2005). The high biocompatibility of apple vinegar is mainly attributed to the high concentration of malic acid in its composition (Caligiani *et al.,* 2007).

Grape vinegar (pH 2.4), like red wine, is rich in polyphenols which are powerful antioxidants. The antioxidants protect the body against the damage done by free radical molecules. Free radicals have been implicated in a number of chronic conditions including cardiovascular disease, cancer and inflammatory conditions. Pomegranate Vinegar (pH 2.93-3.20) also contains polyphenols but at higher levels than other fruit juices and it is the only fruit rich in all three major antioxidants: tannins, anthocyanins, and ellagic acid. Coconut vinegar has a pH 4-5 since coconut trees grow in soil that's highly rich in nutrients and therefore the "sap" from the coconut blossoms is also rich in nutrients. Coconut vinegar is therefore a good source of minerals and vitamins. (Budak *et al.,* 2014)

The purpose of this study is to evaluate and compare in-vitro, by scanning electron microscopy (SEM) the removal of smear layer in the coronal, middle and apical third of root canals irrigated with Apple Cider Vinegar, Palm Vinegar, Pomegranate Vinegar and Grape Vinegar.

MATERIALS AND METHODOLOGY

Fifty freshly extracted permanent human maxillary central incisors with straight root and Vertucci's type 1 root canal anatomy were selected and superficial soft tissues were removed with a brush and all the teeth were stored in distilled water. The teeth were decoronated to standardize root length of 15 mm and the samples were divided into four experimental groups (n=10) and a control group (n=10).

The working length was established by inserting a number 10 K file (Mani Inc.) into each root canal until it was visible at the apical foramen and by subtracting 1mm from this point. Chemomechanical preparation was performed in each tooth using a combination of passive step-back and rotary 0.06 taper nickel titanium files (Dentsply Protaper). The apical foramen of each tooth was enlarged to a size 30K-file. Irrigation was performed with 1ml of 2.5 % sodium hypochlorite solution after enlarging the canal up to a size 30 K-file and further the specimens were divided into five groups. Final irrigation for each group was done using 5ml of each of

the vinegars (Apple Cider Vinegar, Palm Vinegar, Pomegranate Vinegar and Grape Vinegar) respectively followed by 3ml of distilled water.

After irrigation all the root canals were dried with absorbent paper points and a sterile cotton pellet was placed in the access cavity. Longitudinal grooves were prepared on buccal and lingual surfaces of each root using a diamond disc at a slow speed without penetrating the canal. The roots were then split into two halves using a chisel and stored in distilled water at 37° C.

The specimens were dehydrated in a graded series of ethanol solutions, gold sputtered using an ion sputter and immediately examined under scanning electron microscope for the presence or absence of smear layer. Photomicrographs were made at ×1000 magnification randomly at coronal, middle and apical from the thirds of each specimen. Each field was scored according to the following criteria given by Rome *et al.,* :

Score 0 = No smear layer, dentinal tubules open, free of debris.

Score 1 = Root canal surface covered with residue only at the opening of the dentinal tubules.

Score 2 = Root canal surfaces with a thin covering of residue on dentinal tubules with visible tubules only in a few regions.

Score 3 = Heavy smear layer, outlines of dentinal tubules totally covered with smear layer.

STATISTICAL ANALYSIS

The results obtained were used to compare the smear layer removal between the four different groups by ANOVA (Analysis of variance) test followed by Tukey-Kramer multiple comparison test. A p-value less than 0.05 was considered as significant.

RESULT

Removal of smear layer from the surfaces of root canals revealed the presence of more abundant and larger dentinal tubules in the coronal third of root canals compared with those seen in the middle and apical thirds of the root canal system. The dentinal tubules in the apical third of the canals were smaller and fewer than those observed in the rest of the root canals. The greatest amount of smear layer removal was seen by irrigation with apple cider vinegar followed by palm vinegar, pomegranate vinegar and grape vinegar with a p-value = 0.001 which was considered highly significant. Among the coronal, middle and apical third of Group I (ACV), no statistical significance was seen with p-value = 0.329.

Table 1. Showing the scores regarding presence of smear layer in coronal third after irrigation with different solutions

Coronal	Smear layer						Total	Chi square	p
	Root canal surface covered with residue only at the opening of the dentinal tubules		Thin smear layer on dentinal tubules		Heavy smear layer				
	N	%	N	%	N	%			
Apple Cider Vinegar	9	90	1	10	-	-	10	66.13	0.001**
Palm Vinegar	1	10	9	90	-	-	10		
Pomegranate Vinegar	-	-	1	10	9	90	10		
Grape Vinegar	-	-	8	80	2	20	10		
2.5 Sodium Hypochlorite	-	-	2	20	8	80	10		
Total	10	20	21	42	19	38	50		

Table 2. Showing the scores regarding presence of smear layer in middle third after irrigation with different solutions

Middle	Smear layer						Total	Chi square	p
	Root canal surface covered with residue only at the opening of the dentinal tubules		Thin smear layer on dentinal tubules		Heavy smear layer				
	N	%	N	%	N	%			
Apple Cider Vinegar	10	100	-	-			10	66.75	0.001**
Palm Vinegar	-	-	8	80	2	20	10		
Pomegranate Vinegar	-	-	4	40	6	60	10		
Grape Vinegar	-	-	9	90	1	10	10		
2.5 Sodium	-	-	2	20	8	80	10		

Hypochlorite									
Total	10	20	23	46	17	34	50		

Table 3. Showing the scores regarding presence of smear layer in apical third after irrigation with different solutions

Apical	Smear layer						Total	Chi square	p
	Root canal surface covered with residue only at the opening of the dentinal tubules		Thin smear layer on dentinal tubules		Heavy smear layer				
	N	%	N	%	N	%			
Apple Cider Vinegar	8	80	2	20	-	-	10	41.88	0.001**
Palm Vinegar	4	40	6	60	-	-	10		
Pomegranate Vinegar	-	-	3	30	7	70	10		
Grape Vinegar	1	10	8	80	1	10	10		
2.5 Sodium Hypochlorite	-	-	3	30	7	70	10		
Total	13	26	22	44	15	30	50		

Fig 1. Smear layer present only at dentinal tubule openings after irrigation with Apple cider vinegar

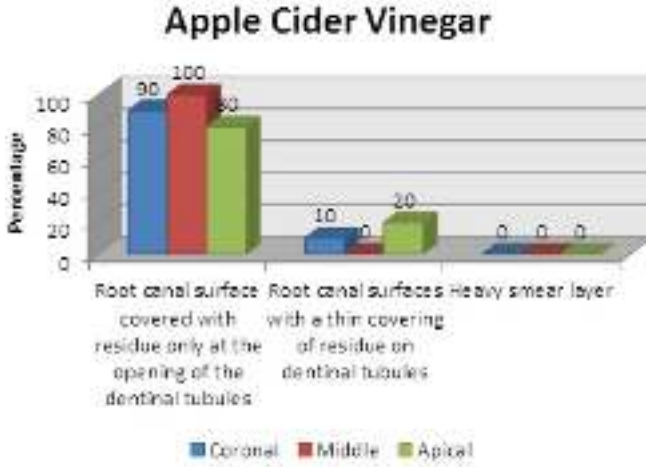


Fig 2. SEM MICROGRAPHS SCORE 1=Root canal surface covered with residue only at dentinal tubule openings.

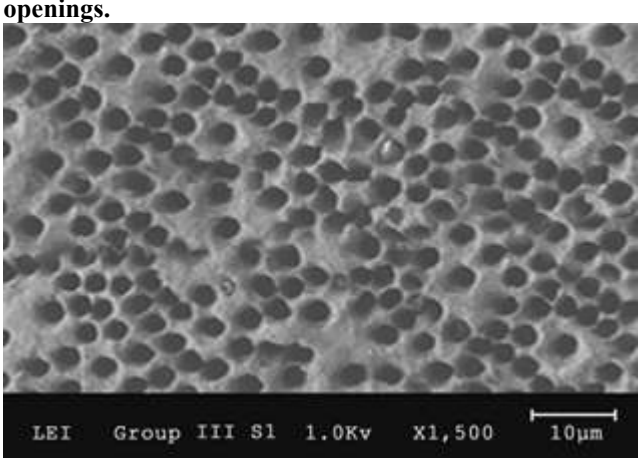


Fig 3. SEM MICROGRAPHS SCORE 2=Moderate smear layer. No smear layer was observed on surface of root canal but tubules contained debris.

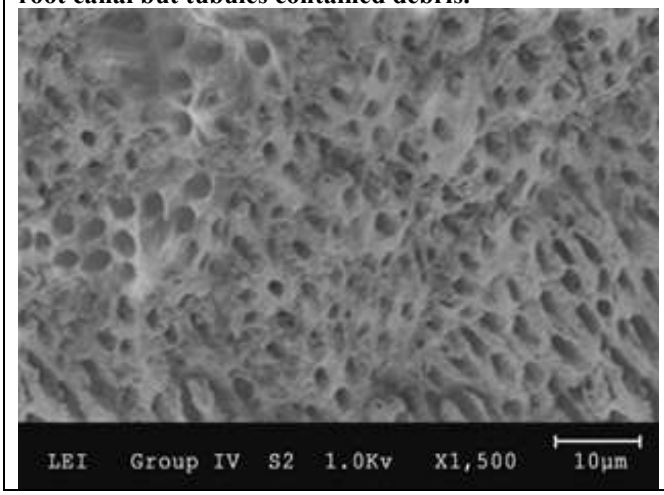
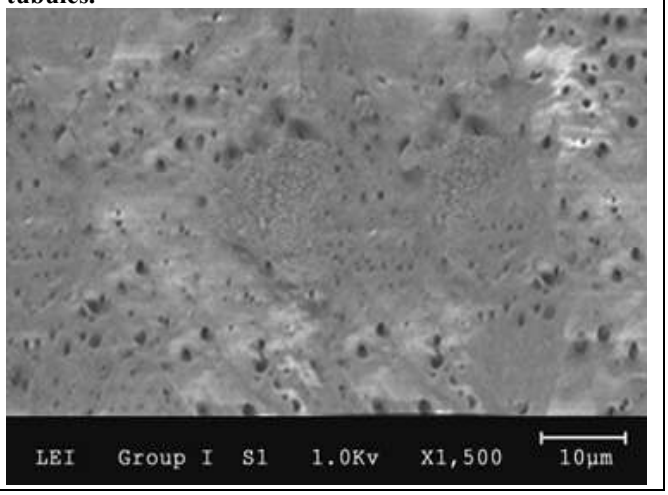


Fig 4. SEM MICROGRAPHS SCORE 3= Heavy smear layer. Smear layer covered the root canal surface and tubules.



DISCUSSION

The main goals of the chemomechanical preparation are to eliminate bacteria and their byproducts from the root canal system, remove pulp tissue remnants and contaminated organic and inorganic debris that are formed during instrumentation and compacted into the dentin tubules and produce a continuously tapered shape in the crown-apex direction to allow effective irrigation and three-dimensional obturation of the canal space. Chemical endodontic irrigants must have some important properties such as biocompatibility, dissolution of organic tissues, bactericidal action and capacity to remove smear layer from the canal walls. Different solutions, such as NaOCl at several concentrations, chlorhexidine and more recently apple vinegar, have been used as endodontic irrigants. (Canderio *et al.,* 2010)

The biocompatibility of apple vinegar is attributed to the presence in its composition of malic acid (Caligiani *et al.,* 2007), which has therapeutic properties. It increases the organism resistance because it is one of the acids of the Krebs cycle, which is a set of reactions responsible for production of energy in the cells. In addition, apple vinegar has a remarkable medicinal potential due to its high mineral content (potassium, phosphorus, magnesium, sulfur, calcium, fluoride and silicon), and contains other elements, such as pectin, beta-carotene, enzymes and amino acids, which attack free radicals that affect the immune system (Estrala *et al.,* 2005) and may have some beneficial role in the periapical repair process. Therefore, it may be assumed that apple vinegar has some antiinflammatory activity, which is an important characteristic for an endodontic irrigating solution. In addition to the biocompatibility, it has been demonstrated that apple vinegar has bactericidal activity against *E. faecalis* (Estrala *et al.,* 2005), which is one of the main microorganisms associated with endodontic treatment failure.

Failure to remove smear layer from the root canal walls is considered as one of the main reasons of endodontic therapy failure (De-Deus *et al.,* 2002). Removal of the smear layer can allow intracanal medicaments to penetrate the dentin tubules in infected root canals more readily and consequently cause a better disinfection procedure. The lack of adherence between the filling material and the smear-covered canal walls compromise the apical seal, which may result in apical leakage, favoring the survival and multiplication of bacteria that were not eliminated during the chemomechanical preparation. (De-Deus *et al.,* 2002; Farhad A and Elahi T, 2004).

Kirchhoff *et al.,* in 2010, compared and assessed the smear layer and calcium ion removal from the root canal using apple vinegar and other chelating solutions. They observed that the higher the concentration of H^+ ions, the more efficient the attack of the acid would be. The concentration of H^+ ions present in the medium is the result of the dissociation constant (K_a). Because the acetic acid is a weak acid, whose K_a is 1.8×10^{-5}; that is, an acid that is little dissociated, it does not have a concentration of H^+ ions that could produce an efficient calcium removal. The larger quantity of calcium ions detected in the malic acid solution is also due to the action of H+ ions. Because the malic acid is a diprotic acid, it has two dissociation constants, $K_{a1} = 3.5 \times 10^{-4}$ and $K_{a2} = 8.0 \times 10^{-6}$. K_{a1} in fact determines the degree of acid dissociation, since the second constant is much smaller (Harris GB, 2001). Since the K_{a1} of malic acid is higher than the K_a of acetic acid, the malic acid dissociates more strongly, with a higher concentration of H^+ ions, promoting removal of calcium ions more intensely.

CONCLUSION

Based on the results obtained and taking into account the limitations of this study, it could be concluded that among the solutions assessed, Apple cider vinegar promoted greater cleaning of the root canal walls and removing a larger quantity of calcium ions compared to the rest. Further studies must be conducted, varying the concentrations and pH of the solutions which may lead to formulating a solution with active agents that favor effective smear layer removal.

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CONFLICT OF INTEREST

No interest

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