The Effect of Apple Vinegar as an Irrigation Solution to Dental Root Canal Microstructure

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Abstract: NaOCl and EDTA are the standard of irrigation solutions. However, long-term use can cause unwanted effects. Apple vinegar is acidic so it can cause demineralization. This research was conducted to determine the effect of apple vinegar as an irrigation solution on the dentin microstructure of the root canals. This research is a laboratory experiment with a posttest only control group design. 24 post-extraction mandibular premolars were divided based on the irrigation solution used, namely 2.5% NaOCl, 100%, 75%, 75%, 25%, 12.5%, 6.25% apple vinegar, and aquadest. The microstructural changes observed with the Scanning Electron Microscope at 2000 magnification. The assessment of cleanliness used the Hulsmann scoring while the erosion was used the criteria of Torabinejad. The cleanliness in the positive control had an average score of 1. The 100% apple vinegar solution group had a score of 1.67. In the lower apple vinegar concentration group had a greater average score. The average dentin erosion scores of each group were compared with the positive control with p-value ≥ 0.05. Apple vinegar as an irrigation solution affects the dentin microstructure of the root canal. There was no erosion, but the cleanliness of the smear layer increased according to the increase of concentration.

1 INTRODUCTION

Root canal treatment is influenced by chemomechanical thorough debridement of the pulp tissue, dentine debris, and microorganisms. Root canal irrigation solution used during root canal treatment provides chemical and mechanical effects. Chemical debridement is indispensable to remove smear layers, especially on teeth with complex internal anatomy or other irregularities that may have been missed by instrumentation during mechanical debridement. The mechanical effect of irrigation is produced by the flow of the irrigation solution during the cleaning and formation of root canals. Irrigation solution can remove debris, dissolving tissues, and disinfect the root canal system. Bacteria are a major etiological factor in the development of pulp and periapical lesions. Research shows that the irrigation solution which has antibacterial properties have better effectiveness.(Borzini et al., 2016; Kirchhoff et al., 2014)

The most effective time to irrigate a root canal is 40 minutes. Ethylene Diamine Tetraacetic Acid (EDTA) and Sodium Hypochlorite (NaOCl) are irrigation solutions widely used in endodontics. Long-term use of EDTA and NaOCl at high concentrations can cause undesirable effects on the root canal wall, such as flexural strength, microhardness, root canal dentin roughness, and significantly reduced elastic modulus. EDTA is used most as a 17% neutralized solution. The EDTA solution reacts with calcium ions in the dentin to form soluble calcium chelates. The pH of the EDTA solution is an important factor that affects the

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cleaning of the root canal walls. NaOCl served as a disinfectant that was widely used in the late 19th century. Organic tissue can react with NaOCl, resulting in saponification, neutralization of amino acids, and can also dissolve necrotic tissue. NaOCl solution is more ideal than other irrigation solutions.(Kaya et al., 2011; Calaletin Topbas & Adiguzel, 2017)

Apple vinegar is made from crushed and extracted apples. Acetobacter bacteria and yeast are added to start the fermentation process. At the beginning of the process, sugar is converted to alcohol and then alcohol is converted into vinegar. The acetic and malic acids in vinegar cause a sour taste and low pH. Apple cider vinegar also contains vitamins B1, B2, B3, B5, B6, B7, B9, pectin, flavonoids, polyphenols, and minerals.(Mohanty et al., 2017; Nazni et al., 2015) In traditional medicine believed apple vinegar has an antibacterial effect. Several studies have examined the bactericidal activity and bacteriostatic. This activity depends on the concentration of acetic acid, incubation time, number of bacteria, and temperature.(Kalaba et al., 2019; Ulbricht & Basch, 2016) Acidic conditions can cause the demineralized dentin.(Kaya et al., 2011; Omar et al., 2016) Demineralization will cause changes in the dentin microstructure. It is necessary to investigate the influence of the dentin microstructure if apple vinegar is used as an irrigation solution.

2 METHODS (AND MATERIALS)

This is experimental laboratory research with a posttest only control group design. The 24 post-extraction of mandibular premolars were planted in wax blocks. The tooth was prepared for the root canals treatment using the crowndown technique. These samples were divided into 8 groups. Each group was given a different irrigation solution. Group I is a positive control will be given 2.5% NaOCl solution. Groups II to VII are the test group. Various concentrations of apple vinegar will be used as an irrigation solution. Group II will be given a 100% apple vinegar solution, group III 75% apple vinegar, group IV apple vinegar 50%, group V apple vinegar 25%, group VI 12.5%, and group VII 6.25%. Group VIII is a negative control, using aquadest as the irrigation solution. The groups can be seen in Figure 1.

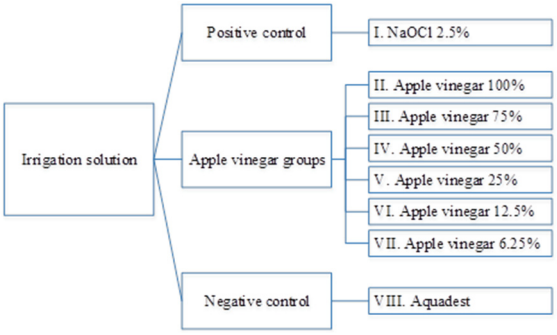


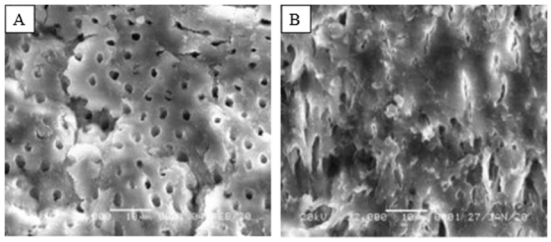
Figure 1: Group category of irrigation solution.

Samples after being treated, the split into 2 halves using a diamond disk and chisel. The apical part was placed on the metal stub, then coated with Pd Au to make it more conductive at the time of observation. The dentin microstructure was observed using a Scanning Electron Microscope (SEM). Taking pictures of the specimen chamber was done with a 2000 magnification. Dentin microstructural changes observed by the cleanliness and erosion in one-third of the root canal. Cleanliness is determined by the presence of a smear layer and the percentage of visible dentinal tubules. The guidelines used are based on the Hulsmann scoring system.(Mancini et al., 2021) Score 1: there is no smear layer and dentinal tubules clearly visible. Score 2: There was a small amount of smear layer and more than 50% open dentinal tubules. Score 3: There is a smear layer almost along the wall of the root canal and dentinal tubules are less than 50% open. Score 4: The entire root canal wall was covered by a smear layer and the dentinal tubules were not clearly visible. The average scoring is then analyzed statistically by non-parametric test Kruskal-Mann continued Whiteney.

The observations of the erosion score for each group was classified according to the criteria of Torabinejad et al. (Görduysus et al., 2015; Torabinejad et al., 2003) Score 0: the smear layer covers almost all the surface of the dentin, little or no open at all. Score 1: no erosion: all tubules appear normal. Score 2: moderate erosion: eroded peritubular dentin. Score 3: severe erosion: the intertubular dentin is crushed and the tubules are connected to each other. ANOVA analysis was used to compare the erosion scores between groups.

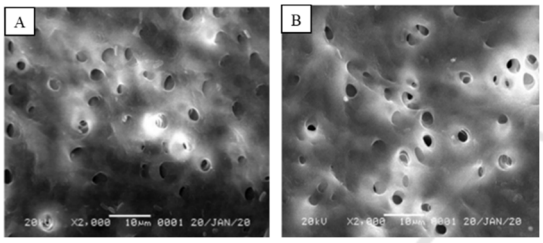
3 RESULTS AND DISCUSSION

SEM images in each group are represented by Figure 2-5. Microstructural changes in each group were observed and categorized based on scoring.



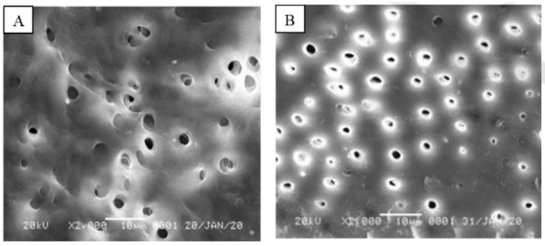
A. Group I, positive control, used 2.5% NaOCl irrigation solution, there was no smear layer, no erosion, the dentinal tubules were clear and normal.
B. Group VIII, negative control, using aquadest, the entire root canal wall was covered by a smear layer.

Figure 2: SEM images (original magnification of ×2000) of representative specimens of group I (A), and group VIII (B).



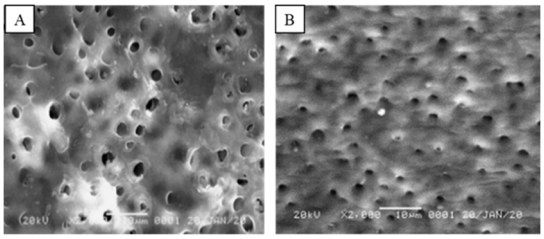
A. Group II, using 100% apple vinegar irrigation solution, no smear layer, no erosion, most of the dentin tubules were open.
B. Group III, using 75% apple vinegar irrigation solution, there was a small amount of smear layer, no erosion, the dentinal tubules looked normal and more than 50% open.

Figure 3: SEM images (original magnification of ×2000) of representative specimens of group II (A), and group III (B).



A. Group IV, using a 50% apple vinegar irrigation solution.
B. Group V, using a 25% apple vinegar irrigation solution.
In both groups, there was a small number of smear layers, no erosion, the dentinal tubules were normal and less than 50% open.

Figure 4: SEM images (original magnification of ×2000) of representative specimens of group IV (A), and group V (B).



A. Group VI, using 12.5% apple vinegar irrigation solution.
B. Group VII, using 6.25% apple vinegar irrigation solution.
In both groups, there was a small number of smear layers, no erosion, the dentinal tubules were normal and less than 50% open.

Figure 5: SEM images (original magnification of ×2000) of representative specimens of group VI (A), and group VII (B).

Cleanliness of the root canal dentin, which is determined by the presence of smear layer and dentin tubules visibility, an average score of 1, in the positive control group, 2.5% NaOCl irrigation solution. Group II, 100% apple vinegar irrigation solution, had an average score of 1.67. The smaller concentration of apple vinegar, the greater the scoring average. The average scoring in each group can be seen in Figure 6.

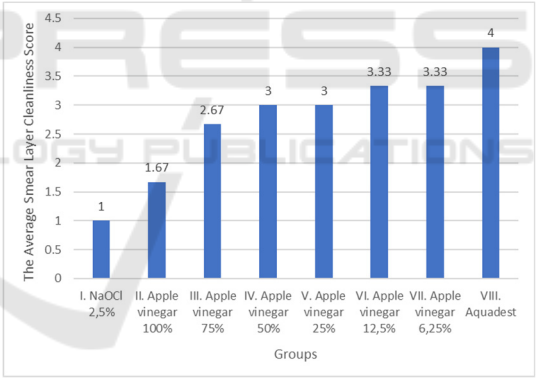


Figure 6: The average score cleanliness of dentin.

The cleanliness smear layer of apple vinegar with 100% concentration had no significant results with positive control. The group of 100% apple vinegar has the same effect as good as 2.5% NaOCl solution. The cleanliness smear layer increased accordingly increased the concentration of apple vinegar.

Erosion microstructure of each group after observed then made the average, as seen in Table 1. The average value highest in the positive control group was 1.42. These data indicate that there is no erosion and dentinal tubules are seen as normal in all test groups.

Table 1: The average score of dentin microstructure erosion.

Test Groups	Average Score	NaOCl 2.5%	Significance
II. Apple vinegar 100%	0,81	1,43	0,858
III. Apple vinegar 75%	0,83		0,257
IV. Apple vinegar 50%	1,08		1,858
V. Apple vinegar 25%	0,75		0,139
VI. Apple vinegar 12,5%	1,42*		0,999*
VII. Apple vinegar 6,25%	0,83		0,257

*Indicates the concentration of apple vinegar, whose erosion is close to NaOCl

The average score after analyzed statistically, a significant difference between groups, with p-value ≥ 0.05. The test group that had a significance value close to NaCl was the 75% apple vinegar group.

The irrigation solution in endodontic treatment has a very important role, such as an antibacterial, lubricating the dentin walls, dissolving the smear layer, and increasing the sealer bonding ability.(Pereira et al., 2012; Calaletin Topbas & Adiguzel, 2017) The use of apple vinegar as an irrigation solution in root canal treatment has good potential.(Kirchhoff et al., 2014) The results of this study are in line with several previous studies regarding changes in dentin microstructure caused by irrigation solutions.(Borzini et al., 2016; Celalettin Topbas & Adiguzel, 2017) The cleanliness and dentin erosion using apple vinegar irrigation solution is similar to NaOCl.

Previous research has found that apple vinegar contains citric, malic, and lactic acids.(Kirchhoff et al., 2014; Mohammadi et al., 2017) Citric acid is a weak organic acid having the molecular formula $C_6H_8O_7$ in the form of a white crystalline powder at room temperature, in anhydrous or monohydrate form. Citric acid has an antibacterial effect.(Dewi et al., 2020)

Citric acid can lower the pH and further suppress the oxidation of nicotinamide adenine dinucleotide (NADH), which resulted in the death of bacteria. Citric acid change the local pH environment that can prevent the absorption of essential nutrients by microorganisms because of the permeability of cell walls which in turn cause damage and cell death, particularly in gram-negative bacteria.(Dewi et al., 2020; Kirchhoff et al., 2014; Mohammadi et al., 2017)

Citric acid also could remove the smear layer. The removal of the smear layer occurs by forming a complex bond (chelate) with the calcium content contained in the inorganic debris of the smear layer. Citric acid is also known as dentin conditioning, its dentine conditioning properties can dissolve dentin hydroxyapatite so that the collagen fibril tissue will open. Citric acid is 10% better than ultrasound irrigations for removing the smear layer from the root apex.(Gutmann et al., 1994)

Apart from citric acid, malic acid with the molecular formula $C_4H_6O_5$ is the main acid found in many fruits, including apples, apricots, blackberries, blueberries, cherries, grapes, mirabella, peaches, pears, and plums which were first obtained from apple juice by Carl. Wilhelm Scheele in 1785.(Setyawati & Nur, 2020)

Malic acid is a carboxylic acid group that could whiten teeth by oxidizing the surface of the tooth enamel to become neutral and causing a whitening effect. Malic acid has a very low molecular weight so that it can diffuse into enamel and dentin, and can oxidize the surface of tooth enamel by releasing oxygen-free in the double bonds of organic and inorganic compounds in teeth.(Kirchhoff et al., 2014)

Lactic acid with the molecular formula $CH_3CHOHCOOH$ is often associated with dental caries. The use of lactic acid with a concentration of 20% applied for 10 seconds will produce a demineralized surface, while at a concentration of 30% it will remove the smear layer, increase the diameter of the dentinal tubules, and affect the collagen matrix (Kirchhoff et al., 2014).

4 CONCLUSIONS

Apple vinegar as an irrigation solution affects the dentin microstructure of the root canals. There was no erosion, but the cleanliness of the smear layer increased according to the increase in the concentration of apple vinegar.

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