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Research Article

EFFECT OF GRAPE SEED EXTRACT SOLUTION ON THE MICROHARDNESS OF THE ROOT CANAL DENTIN: AN *IN VITRO* STUDY

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ABSTRACT

Objective: Grape seed extract (GSE) containing proanthocyanidin as a root canal irrigation solution has its antibacterial effects and ability to eliminate the smear layer. In addition, proanthocyanidin acts as a cross-linking agent of collagen, which adds to dentin's mechanical properties. This study analyzed the effect of GSE containing 2.9% proanthocyanidin on the microhardness of the dentin in the root canal.

Methods: Fifty teeth were divided into three groups and immersed in GSE solution, 3% NaOCl solution, or distilled water (controls) for 30 min for 3 consecutive days. The microhardness was measured using the Vickers method. Data were analyzed using the Kruskal–Wallis test.

Results: The GSE group presented the highest microhardness values, whereas the lowest values in the NaOCl group. No significant difference in microhardness observed between the GSE and distilled water groups.

Conclusion: The GSE solution maintains the microhardness of the root canal dentin.

Keywords: Grape seed extract, Proanthocyanidin, Microhardness, Root canal dentin.

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INTRODUCTION

One of the primary purposes of endodontic treatment is hermetic obturation. A tight apical seal prevents the entry of bacteria and its products into the root canal. Low-quality root canal obturation increases the risk of microleakage, which results in endodontic treatment failure; apical microleakage is the most common cause of endodontic treatment failure. The method used to obturate the root canal influences the occurrence of the apical leakage, the formation of the smear layer, and the adhesion of the root canal filling material [1].

The chemical irrigants commonly used in endodontic treatment have certain disadvantages because they can alter the composition of the dentin's inorganic and organic contents, mainly collagen. Type I collagen is the main constituent of the organic structure of the dentin. It acts as a scaffold and forms a framework for the mineralized material, determining the dentin's mechanical properties [2]. Changes in the inorganic structure of the dentin and the components of the root canal due to the use of chemical irrigation solutions can increase the risk of vertical root fractures in post-endodontic teeth [3]. Alterations in the organic and inorganic components of the dentin seen in scanning electron microscope images as cracks in the intertubular dentin after irrigation with EDTA (17%) and NaOCl (2.5%) [4]. Besides, the damaged dentin collagen interferes with the formation of a hybrid layer that can affect the adhesion of the root canal sealant to the dentin [5]. One of the mechanical properties which decreased after the use of the root canal irrigation solution was microhardness – an indication of the decrease in the condition of the collagen. The damaged condition of the collagen in the root canal can reduce the adhesion of root canal sealer to the dentin, leading to microleakage and root canal failure. As a result, studies on the use of natural materials as antibacterial root canal irrigation solutions that did not reduce dentin's mechanical properties have been conducted [5,6].

Prevention of damage to dentin's collagen by the formation of collagen bonds is more stable and resistant to biodegradation. The application of a collagen cross-linking agent can maintain collagen stability. One of the natural collagen cross-linking agents that have recently studied is a component of grape seed extract (GSE) called proanthocyanidins [7,8]. GSE solutions have been shown to have antibacterial properties and can eliminate the smear layer [9,10]. Furthermore, GSE has shown to have a positive effect on some of the mechanical properties of dentin, such as flexural strength and microhardness [6].

However, GSE's effect on the microhardness of the dentin in the root canal has not evaluated so far. This study aimed to analyze the effect of GSE (containing 2.9% of proanthocyanidin) on the microhardness of the root canal dentin.

MATERIALS AND METHODS

Specimens

Until the experiment starts, 50 human single root premolars extracted for orthodontic purposes stored in saline solution. The apical portions of the teeth prepared with a diamond disk and chisel under water spray. The apical third of the tooth was sectioned into 5 mm dentin disks in a mesiodistal direction, parallel to the longitudinal axis of the tooth. All the samples rinsed with saline solution, and each dentin disk embedded in an acrylic resin block with the inner side of the root canal exposed. All samples stored in a closed container with a saline solution to maintain dentin hydration until further use [11,12].

Groups

The root canal dentin disks randomly divided into three groups based on the irrigation solution they immersed in: Group 1 (n=30) immersed in GSE with proanthocyanidin (2.9%), Group 2 (n=30) immersed in sodium hypochlorite (NaOCl; 3%), and Group 3 (controls: n=30) immersed in distilled water. Each sample immersed in 5 mL of irrigation solution, 30 min per day for 3 consecutive days [13,14].

Measurement

The microhardness measurement uses the Vickers method with 500 g per 15 s indentations [14]. Three indentation points measured in each sample. The measurements took at the Balai Besar Teknologi Kekuatan Struktur (Center for Structural Strength Technology), Pusat Penelitian Ilmu Pengetahuan dan Teknologi (Research, Science and Technology Center), South Tangerang, Indonesia.

Statistical analysis

The data were tabulated and statistically analyzed using the Kruskal–Wallis and *post hoc* Mann–Whitney U tests. The level of significance was 5%. All data were analyzed using IBM SPSS Statistics Software, version 20 (IBM Corp., Armonk, NY, USA)

RESULTS AND DISCUSSION

Root canal dentin microhardness value

The highest median microhardness observed in Group 1 (GSE group) followed by Group 3 (distilled water) and Group 2 (NaOCl; Table 1). Likewise, the highest average value of dentin root canal microhardness was Group 1 followed by Group 3 and Group 2. The highest microhardness value measured in the 2.9% GSE group was 32.21±15.14 HV, and the lowest value in the 3% NaOCl group was 21.24±9.23 HV. No significant difference in microhardness value noted between Group 1 and Group 3 (controls).

Irrigation solution influence to dentin's microhardness

Chemomechanical preparation is one of the most critical factors that determine the success of root canal treatment. The 3% NaOCl irrigation solution commonly used for endodontic treatment has antibacterial activities and dissolves the necrotic tissue in the root canal [15]. However, it alters the composition of the organic and inorganic structures of the tooth [13,14]. Moreover, it is toxic to the tissues due to its chemical nature [16]. Therefore, various studies evaluating the potential of natural materials for use as root canal irrigation solutions have been conducted [5,6,9,10,17,18].

GSE is one such natural ingredient, which contains a high proportion of proanthocyanidin. GSE solution has shown to eliminate the root canal smear layer and act against *Enterococcus faecalis* [9,10]. Another advantage of GSE is the ability to increase collagen cross-linking, which can improve the quality of the organic and inorganic tooth structures, thus increasing dentin's mechanical properties [5,6,17].

Dentin microhardness, one of the dentin's mechanical properties, is influenced by the amount of collagen matrix and mineral component in the dentin. Collagen acts as a scaffold for mineralized material in the dentin, resulting in the association between the amount of dentin collagen matrix and minerals contained in dentin. As the amount of collagen matrix formed on the dentin increases, the mineral component also increases. The increase in dentin microhardness indicated by an increase in the number of calcified matrices per mm^2. Therefore, microhardness measurements have commonly used to demonstrate the loss or increase of minerals in hard tooth tissues, including dentin [19,20].

Changes may occur in the organic and inorganic components of the dentin in post-endodontically treated root canals. One such change is the decrease in microhardness, commonly caused by the irrigation solutions used for endodontic treatment [13]. The decrease in microhardness provides evidence of the reduction in the dentin's inorganic components and an overview of the dentin collagen matrix's condition. These changes result in the formation of cracks in the intertubular dentin, which will reduce the resistance of the root canal wall and increase the risk of fractures in the endodontically treated teeth [4].

Table 1: Value of root canal dentin microhardness after the experiment

Experimental groups	Median (min–max)	Mean±SD	p-value
2.9 % GSE	27.04 (9.92–63.34)	32.21±15.14	0.002
3 % NaOCl	19.75 (6.83–43.88)	21.24±9.23	0.002
Distilled water	26.31 (8.86–59.14)	29.55±12.63	0.002

Damage to the dentinal collagen after the use of the NaOCl irrigation solution disrupts the formation of a hybrid layer due to the formation of an oxygen-rich layer on the dentin; this eliminates the organic matrix (collagen) and exposes the dentin surface, thus affecting the seal against the dentin [5]. The adhesion of the root canal sealer affects the quality of the root canal obturation. It determines the success of the treatment [1].

Proanthocyanidin in GSE as cross-linking agent

GSE contains proanthocyanidin, which is known to have antibacterial properties, can eliminate smear layers, and improve dentin's mechanical properties. Proanthocyanidins can induce the cross-linking of collagen molecules in the dentin by increasing the number of inter- and intra-molecular collagen cross-links, thus strengthening the dentin collagen matrix and increasing the stability of the collagen by preventing enzymatic degradation, which in turn will improve the mechanical properties of dentin [5,17].

Proanthocyanidin molecules consist of multiple free phenyl hydroxyl groups that contain free hydroxyl groups, which can form bridge-type hydrogen bonds with the side chains of hydroxyl, carboxyl, amino, and collagen molecular amides. The formation of hydrogen bonds causes the stability of proanthocyanidin-collagen interactions. Furthermore, because of its position between the collagen molecules, proanthocyanidin can form ionic and covalent bonds with collagen fibrils [21]. During the process of forming hydrogen bonds, proanthocyanidin molecules can replace water molecules that bind to collagen in the extra fibrils [21]. This results in cross-linking and the formation of more stable collagen, thus enabling the maintenance of the mineral component and microhardness of the root canal dentin.

The GSE (*Vitis vinifera*) used in this study originates from Turkey and has used to prepare solutions for healthy drinks. The majority of the local grape varieties cultivated in Indonesia belong to this type [22]. The climate conditions in the lowlands with the hot weather and loose sandy soil in Indonesia, especially in Bali, Kediri, and Probolinggo, are suitable for grape cultivation. The weather does not affect the quality of the wine originating from Indonesia, as well as the quality of the wine from Europe and Central Asia [23,24].

The local GSE is processed based on the concentration of GSE in the solution. According to Angellina (2013), the GSE solution's concentration did not affect the ability to clean the smear layer in the root canal [9]. GSE is an irrigation solution that cleanses the smear layer not based on the GSE concentration. However, the role of GSE in the cross-linking of the collagen influenced by the concentration of proanthocyanidin. Based on several previous studies, 3.75% and 6.5% of proanthocyanidin in GSE can significantly increase collagen cross-linking [25,26]. Additional studies are required to evaluate the effects of various concentrations of proanthocyanidin in GSE on the dentin in the root canal.

Application of irrigation solution

The 3% NaOCl solution in this study based on a previous study by Ghonmode *et al.* (2013) compared the antibacterial effect of GSE with 3% NaOCl on *E. faecalis* [18]. Similarly, Kalluru (2014) demonstrated the effects of several commonly used irrigation solutions on the dentin's microhardness in the root canal [11]. Antibacterial effect and ability to dissolve pulp and necrotic tissue at a concentration of 3% is superior to those at concentrations of 0.5% and 1%. Higher concentrations of NaOCl will exert more toxic effects on the periapical tissue [27].

Until now, there has been no standard regarding the ideal duration of root canal irrigation. Based on an *in vitro* study by Retamozo *et al.* (2010), the best effect of irrigation with 5.25% NaOCl observed for 40 min. If a lower concentration of 1.3% or 2.5% used, the duration of 40 min is not sufficient for removing *E. faecalis* from the dentinal tubules [28]. In this study, the canal root canal disk was immersed in the test solution for 3 consecutive days, with 30 min of immersion time per day. Based on the clinical simulation of root canal treatment duration, the root canal was exposed to irrigation solutions for 30 min every appointment.

Several studies on the use of GSE as a root canal irrigation solution have employed different application methods. In the study by Cecchin (2015), dentin plates were soaked for 40 min in the test solution to compare the effect of 2.5% NaOCl and 6.5% GSE on the dentin's flexural strength [6]. Whereas in the study by Manimaran (2011), GSE's effect with 5% proanthocyanidin on the bonding strength of the sealer to the dentin after root canal irrigation with 5.25% NaOCl evaluated; the GSE solution used as a final irrigation solution for 10 min [5].

Further research evaluating the effects of various durations of application of the solution is warranted. Exposure to the irrigation solution was related to variations in the endodontic treatment time by different clinicians. Thus, by adding the time of application of the test solution as a variable, the most effective duration of GSE solution as irrigation can be discovered.

Microhardness measurement method

Several methods used to measure microhardness, and the two most common methods are the Vickers and Knoop methods [13]. In this study, the Vickers method used because it can evaluate surface changes in the deeper hard tissues of the teeth, whereas Knoop hardness testing more commonly used for surface dentin (depth, 0.1 mm) [13]. The microhardness value obtained on the dentinal root canal plate after being exposed to a load of 500 g in 15 s has determined by its indentation point [14].

The dental root canal plate used in this study originated from the apical one-third of the dentin root canal. This section chooses because the density of this area after a root canal filling is one of the determinants of the success of the treatment [29]. Microhardness is one of the factors that influence the level of sealer adhesion obturation in the dentin root canal.

GSE solution effect to dentin's microhardness

The results of the statistical tests in this study (Table 1) show that there are significant differences ($p=0.002$; $p<0.05$) in the dentinal root canal microhardness values among the GSE (32.21±15.14), NaOCl (21.24±9.23), and distilled water (29.55±12.63) groups. Thus, both GSE with a proanthocyanidin content of 2.9% and 3% NaOCl influenced the dentin root canal microhardness. The microhardness of the GSE group was higher than those of the NaOCl and distilled water groups. The microhardness value of the GSE group showed higher results compared to the NaOCl group ($p=0.002$). Likewise, the GSE group's microhardness was higher than that of the distilled water group, statistical significance notwithstanding ($p=0.631$). The 3% NaOCl solution can reduce dentin root canal microhardness, while the GSE solution with proanthocyanidin (2.9%) is not proven to be able to improve the microhardness. The mean value of dentin root canal microhardness in the GSE group (32.21±15.14) was higher than that of the distilled water group (29.55±12.63), although the difference was not statistically significant ($p>0.05$). Thus, it may conclude that GSE can maintain the microhardness of the dentin root canal.

NaOCl (3%) solution has shown to significantly reduce the microhardness of the dentin in the root canal [11]. The microhardness in the NaOCl group (21.24±9.23) was lower than those in the distilled water (29.55±12.63) and (32.21±15.14) GSE groups. These findings clearly show that 3% of NaOCl can reduce dentin's microhardness in the root canal.

The role of proanthocyanidin in GSE

In this study, the role of proanthocyanidin in GSE as a cross-linking agent of collagen, which can increase dentin microhardness, did not differ significantly compared with distilled water control solution. This condition possibly caused by the content of glycerin in the GSE solution used. Glycerin as a thickener increases the GSE solution's surface tension so that the penetration into the dentinal tubules will be lower. Therefore, the role of proanthocyanidin molecules in GSE solution with glycerin could not be optimum to increase the microhardness of dentin in the root canal. In a previous study by Liu *et al.* (2015), the small molecular size of proanthocyanidin reported providing the best results in triggering an increase in dentin collagen's mechanical properties [30].

The microhardness evidenced by mineral loss or an increase in the hard tooth tissue and indirectly illustrates the extent of collagen matrix bonding in the dentin. Dentin microhardness is determined by the strength of the collagen matrix bonding to the dentin and the number of calcified matrices per mm^2 [19]. The collagen matrix's amount and stability in the dentin root canal will affect the strength of the adhesion of the resin-based sealer to the dentin. Application of GSE with 5% proanthocyanidin shown to increase the adhesive strength of the resin to a root canal that irrigated with 5.25% of NaOCl [5].

Proanthocyanidin molecules consist of free hydroxyl groups that form bridge-type hydrogen bonds with the side chains of hydroxyl, carboxyl, amino groups, and collagen amides. These hydrogen bonds account for the stability of the proanthocyanidin-collagen interactions. Furthermore, due to its position between the collagen molecules, proanthocyanidin can form ionic and covalent bonds with collagen fibrils. The proanthocyanidin molecules replace the water molecules that bind to collagen in the extra fibrils to form these hydrogen bonds.

Proanthocyanidin stabilizes and enhances the cross-linking of type I collagen fibrils by proline hydroxylation, an essential stage of collagen biosynthesis. Thus, the collagen cross-links are more stable in the dentin matrix. Intermolecular cross bonds determine the stability, strength, and viscoelasticity of the collagen fibrils. Collagen fibrils reinforced by cross-linking agents such as proanthocyanidin will have increased mechanical properties and reduced enzymatic degradation, which plays essential roles in material adhesion to the dentin [2].

CONCLUSION

The findings of this study show that GSE can increase the dentin's microhardness in the root canal. GSE solution can be used as an irrigation material during root canal treatment, and a solution containing 2.9% of proanthocyanidin can maintain the microhardness of the root canal dentin.

Further research is needed to evaluate the microhardness of the root canal using GSE in conjunction with various irrigation techniques and durations; also, the effect of local GSE from Indonesia containing various concentrations of proanthocyanidin needs evaluation.

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AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

Declared none.

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