QUERCETIN AS AN ENDODONTIC IRRIGANT: EFFECT OF TWO CONCENTRATIONS ON DENTIN MICROHARDNESS

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ABSTRACT

Aim: The aim of this study was to evaluate the effect of two concentrations of quercetin irrigant (2% and 6.5%) and 2% chlorhexidine (CHX) irrigant on root canal dentin microhardness.

Materials and methods: The study was done on thirty human extracted teeth. Their crowns were removed at the cementoenamel junction (CEJ). They were categorized randomly into three groups in accordance with the type of irrigant used. Each group contained ten teeth. Group A: 2% quercetin irrigant. Group B: 6.5% quercetin irrigant. Group C: 2% CHX irrigant. The roots were sectioned in a longitudinal direction by a chisel into two halves. Each half was dipped in an acrylic resin. One half was used as a control to measure the microhardness without irrigation, and the other half of the same root was used as an experimental half to measure the microhardness after the application of irrigants for fifteen minutes. Microhardness was measured by a Vickers tester.

Results: 6.5% quercetin irrigant (group B) was significantly the highest mean value of microhardness, followed by 2% quercetin irrigant (group A), while 2% CHX irrigant (group C) was significantly the lowest mean value of microhardness in all sections and overall.

Conclusion: The microhardness of root canal dentin was increased after irrigation with 6.5% quercetin irrigant and also after irrigation with 2% quercetin irrigant, but to a lesser extent, whereas it was decreased after irrigation with 2% CHX irrigant.

KEYWORDS: Chlorhexidine, Irrigation, Microhardness, Quercetin
INTRODUCTION

All endodontic specialists seek to obtain complete success in root canal treatment cases, and this is achieved through a combination of proper cleaning and shaping procedures. However, they face many obstacles, such as accessory canals, complicated root anatomy, ramifications, and bacterial biofilms (1).

Irrigating solutions are an important factor in the success of root canal treatment. An ideal irrigant must meet the following conditions: has the ability to remove smear layer or dentin debris; has antimicrobial and anti-inflamatory properties; biocompatible with periapical and periodontal tissues; has lubrication properties; does not retard the setting of sealer or obturation materials; affordable; does not alter the morphological structure of dentin or affect its microhardness; simple to use. Most irrigating solutions that are used during root canal treatment have some of these properties, but so far, there is no one that has all of them (2).

Chlorhexidine (CHX) irrigant was widely used during endodontic treatment in concentrations ranging from 0.12% to 2% (3). It has many advantages, such as antibacterial properties, substantivity, and low cytotoxicity that qualify it to be one of the most frequently used root canal irrigants (4). Furthermore, it has the ability to permeate deeply through dentinal tubules, and this is due to its low surface tension (5). Its antibacterial properties are due to the fact that it has a positive charge and can interact with bacterial cells with a negative charge, leading to the breaking down of its cell membrane (6, 7). However, it has some limitations, such as less antibacterial efficacy against gram-negative bacteria and the inability to remove necrotic remnants or the smear layer (8). In addition, some researchers have shown the occurrence of allergic reactions in some patients (9).

Therefore, efforts to find the perfect irrigants for endodontic therapy are constantly being made. Recently, due to a number of benefits of natural products, such as convenience of use, abundant material sources, fewer side effects, and higher efficacy, researchers have shown a strong interest in studying natural products to ascertain their efficacy for various dental objectives (10, 11).

Flavonoids, which are organic pigments with varying phenolic structures, can be found in vegetables, cereals, fruits, roots, flowers, stems, and tea. They can be divided into four primary categories: flavanones, anthocyanins, flavones, and catechins (12).

Quercetin, a flavonoid that has been the subject of extensive research, belongs to this category. Broccoli, onions, berries, tea, and apples have large amounts of it (13). Due to its capacity to eliminate reactive oxygen species and superoxide anions, it offers a wide range of biological and pharmaceutical advantages, including antioxidant, anti-inflammatory, antiviral, and anti-allergenic capabilities (14). Additionally, it has been demonstrated to be a powerful antibacterial agent against a variety of pathogenic microbes, including Enterococcus faecalis (15-17). The specific mechanism of its antibacterial effect is yet unknown, although it most likely involves membrane rupture and irreversible complex formation to inactivate extracellular proteins of pathogenic microbes (18).

Any newly launched products must have their material qualities properly determined before use (19). Root canal dentin is exposed to irrigant solutions during endodontic treatment, which may have an impact on the structural characteristics of dentin such as permeability, microhardness, and solubility, which can change the ratio of organic and inorganic components. In consequence, a decrease in dentin microhardness is regarded as a sign of indirect dentin mineral alterations (20).

Therefore, the aim of this study was to evaluate the effect of two concentrations of quercetin irrigant (2% and 6.5%) and 2% CHX irrigant on root canal dentin microhardness.
The null hypothesis of our study was that the three tested irrigants had the same effect on the microhardness of root canal dentin.

MATERIALS AND METHODS

Sample size calculation
It was calculated depending on Aslantas et al. (21) as a guide. This study determined that the smallest acceptable sample size for each group was 10 samples when the mean (± standard deviation) was 62.86 ± 1.57, the true mean of the other group was 65, when the effect size was 1.36, with the power 80% and type I error probability was 0.05.

Ethical approval
This study was approved by Fayoum University Supreme Committee for Scientific Research Ethics (FU-SCSRE) and the approval code was EC 2357.

Selection of the samples
This study was done on thirty extracted human teeth taken from patients who had their teeth extracted for orthodontic purposes in the surgical clinic at the Faculty of Dentistry of Fayoum University. The chosen teeth fulfilled the following criteria: single root, mature apex, with no cracks or fractures. They were stored in saline solution after being cleaned of any soft tissue remnants or calculus by an ultrasonic scaler.

Preparation and classification of the samples
A tapered diamond stone was used to remove the crowns of the extracted teeth at the cementoenamel junction (CEJ). The length of the roots was set to 15 mm (±) 2 mm length. They were categorized randomly into three groups in accordance with the type of irrigant used. Each group contained ten teeth.

- Group A: 2% quercetin irrigant
- Group B: 6.5% quercetin irrigant
- Group C: 2% CHX irrigant

Fluting was done in all roots on the distal and mesial surfaces of the root canals from the outward direction, taking into consideration not to prejudice the inside surface of the roots. It was done by a low-speed diamond disc. Then the roots were sectioned in a longitudinal direction by a chisel into two halves, resulting in sixty halves. Each half was dipped in an acrylic resin (Acrostone, Egypt) horizontally so that the outer surface of the root was facing the acrylic resin and the inner surface was facing upward. Fine polishing strips were used to smooth the exposed dentin surface.

One half was used as a control to measure the microhardness without irrigation, and the other half of the same root was used as an experimental half to measure the microhardness after irrigation.

Preparation of quercetin irrigants
The 2% and 6.5% quercetin irrigants were prepared by dissolving 2 g and 6.5 g of pure quercetin powder (Nanogate Company, Cairo, Egypt) in 100% ethanol, respectively, then the solutions were placed in a 37 °C water bath for 15 minutes.

Application of irrigants
According to each group, group A: 2% quercetin, group B: 6.5% quercetin, and group C: 2% CHX, 2 ml of irrigant was applied to the exposed dentin surface of the experimental halves for fifteen minutes at room temperature. Then all samples were rinsed with 10 ml of distilled water and dried.

Evaluation of dentin microhardness
Microhardness was measured for the control and experimental halves. It was measured by a Vickers tester (Model LM-100, FM 1159 LECO Corporation Michigan, and USA) at a magnification of X 100 using a 25 g load for 10 seconds. The measurements were taken at three points: apical, middle, and cervical. Three values were taken for each segment, and their average value was recorded.

Statistical analysis
SPSS 16® (Statistical Package for Scientific Studies), GraphPad Prism, and Windows Excel
were used in performing the statistical analysis. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to investigate the given data for normality, and they showed that the data originated from normal data. To compare three different groups, a One-way ANOVA test was used, followed by Tukey’s Post Hoc test for multiple comparisons, while a Paired t-test was used to compare the control and experimental samples. The significance level was set at $p \leq 0.05$.

RESULTS

Comparison between control samples and experimental samples in all groups

The mean values of microhardness of the control samples were statistically significantly lower than the mean values of microhardness of experimental samples for the cervical, middle, and apical thirds and overall in both 2% quercetin irrigant (group A) and 6.5% quercetin irrigant (group B), while in 2% CHX irrigant (group C), control samples were statistically significantly higher in mean values of microhardness than experimental samples for the cervical, middle, and apical thirds and overall ($P < 0.0001$), as shown in table (1) and figure (1).

Comparison between groups A, B, and C

In the control samples, there was no statistically significant difference between the mean values of microhardness in all groups as $P>0.05$ in all sections and overall.

In the experimental samples, 6.5% quercetin irrigant (group B) was statistically significantly the highest mean value of microhardness, followed by 2% quercetin irrigant (group A), while 2% CHX irrigant (group C) was statistically significantly the lowest mean value of microhardness in all sections and overall.

In the difference between the control samples and the experimental samples, the 6.5% quercetin irrigant (group B) was statistically significantly the highest mean value of microhardness, followed by 2% quercetin irrigant (group A), while 2% CHX irrigant (group C) was statistically significantly the lowest mean value of microhardness in all sections and overall, as shown in table (1) and figure (1).

TABLE (1): Mean and standard deviation of cervical, middle, apical thirds and overall, in all groups and comparison between all groups:

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>SD</th>
<th>M</th>
<th>SD</th>
<th>M</th>
<th>SD</th>
<th>P value</th>
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<tr>
<td></td>
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<td></td>
<td>Group B</td>
<td></td>
<td>Group C</td>
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<tr>
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<td>0.900</td>
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<td>0.71</td>
<td>64.99</td>
<td>0.87</td>
<td>53.44</td>
<td>1.09</td>
<td>&lt;0.0001*</td>
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<td>7.09</td>
<td>0.21</td>
<td>-4.38*</td>
<td>0.38</td>
<td>&lt;0.0001*</td>
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<td>0.87</td>
<td>57.79*</td>
<td>1.06</td>
<td>57.77*</td>
<td>1.01</td>
<td>0.91</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
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<td>0.91</td>
<td>57.43*</td>
<td>1.14</td>
<td>57.58*</td>
<td>1.06</td>
<td>0.76</td>
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<td>0.71</td>
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<td>1.10</td>
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<td>0.23</td>
<td>7.04</td>
<td>0.16</td>
<td>-4.38*</td>
<td>0.34</td>
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<tr>
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</tr>
<tr>
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<td>57.72*</td>
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<td>0.880</td>
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<td>53.36</td>
<td>1.10</td>
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<td>-4.36*</td>
<td>0.31</td>
<td>&lt;0.0001*</td>
</tr>
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</table>

*M: mean  SD: standard deviation  *Significant difference as $P<0.05$.
Means with different superscript letters were significantly different as $P<0.05$.
Means with the same superscript letters were insignificantly different as $P>0.05$. 

DISCUSSION

The main goal of endodontic therapy is to reduce bacteria, which supports the natural repair of the periodontal tissues. Endodontic irrigants are crucial in keeping the root canals clean during the biomechanical preparation of teeth with periapical diseases (22). Since resistant microorganisms have been linked to the failure of adequately cleansed and shaped root canals, the efficacy of endodontic irrigants is typically evaluated by their antimicrobial activity against resistant microbes like Enterococcus faecalis (23). Recently, endodontists have employed herbal remedies to avoid the cytotoxic effects of many commonly used irrigants and to be able to get rid of microorganisms that are specifically located inside the dentinal tubules of root canal systems. The focus of modern medicine has been on using plant extracts (24).

Before implementing new irrigation, laboratory research must be conducted to examine the advantages and drawbacks. Hence, this study aimed to evaluate the effect of two concentrations of quercetin irrigant (2% and 6.5%) and 2% CHX irrigant on root canal dentin microhardness.

Although numerous endodontic irrigants have been explored, some exhibit a number of negative consequences, including decreased effectiveness, allergenic possibility, cytotoxicity to cells, and even a decrease in the mechanical qualities of dentin (25). Endodontic irrigants should be biocompatible with human cells and tissues since they come into close touch with them in clinical settings. It is well known that quercetin has low cytotoxicity and outstanding biosafety (26). Because of its many beneficial qualities, we think quercetin has a lot of potential for use in dental applications. For instance, increasing the thickness and length of the root canal wall and removing infection from the root canal and apical area are both necessary for the success of regenerative endodontic procedures (REP), which suggests quercetin’s potential use as an auxiliary root canal irrigant for REP (27). Furthermore, several pathogenic bacterial infections can be prevented and treated with the help of quercetin’s broad-spectrum antibacterial capabilities, which also offer potential alternatives to antibiotic therapy. It is anticipated that quercetin will develop into a novel medication through ongoing research that can both prevent and treat a number of disorders (28).

In our study high concentration of quercetin (6.5%) was chosen as Liu et al. (26) proved that the higher the concentration of quercetin irrigant, the greater the antimicrobial effect and the deeper permeation through dentinal tubules. Application of both concentrations as well as CHX was done at room temperature; as all study irrigants are not temperature-sensitive, and several similar studies have not specified a temperature for testing them (4, 11, 29).

As CruzFilho et al. (30) stated longitudinal root sectioning can display an accurate representation of clinical conditions; hence, it was chosen for the current investigation instead of transversal root sectioning. The microhardness test is an easy and non-invasive technique for assessing how substances affect a substrate, and it indirectly assesses the acquisition or loss of dentin minerals following particular operations (31). Dentin’s microhardness can differ significantly between teeth; hence, in the current investigation, after sectioning each
One half was used as a control to measure the microhardness without irrigation, and the other half of the same root was used as an experimental half to measure the microhardness after irrigation to obtain a realistic assessment of the irrigant solutions’ impact on the dentin surface (32).

Two testing techniques are typically used to determine the microhardness of root dentin: Knoop microhardness testing and Vickers microhardness testing. Instead of using Knoop testing in this work, Vickers microhardness testing was used since it is more sensitive to measurement mistakes, less sensitive to surface circumstances, and can more accurately evaluate small specimens (33).

The results of the study stated that 6.5% quercetin irrigant showed a statistically significant increase in the microhardness of root canal dentin, followed by 2% quercetin irrigant in the cervical, middle, and apical thirds and overall. These findings could be explained by quercetin’s high concentration of phenolic hydroxyl groups, which interact with the collagen of root dentin through van der Waals force, hydrophobic force, hydrogen bond, and electrostatic force. This interaction would maintain the collagen matrix’s bio-stability after irrigation and enhance the mechanical properties of dentin (34).

Also, hydroxyl groups form complexes with calcium ions in dentin, resulting in the deposition of minerals on the surface of dentin, which acts as nucleation areas for hydroxyapatite crystals (35). Furthermore, quercetin increases collagen’s resistance to enzymatic breakdown. It does this by inhibiting both free and collagen-bound proteolytic enzymes in dentin as well as by down-regulating endogenous protease production (36) to render the protease inactive and by blocking collagenase’s access to locations containing collagen chains (37,38). Additionally, quercetin forms crosslinks with the exposed dentin to protect the remaining dentin from acid assault by creating a mechanical barrier to the collagen matrix (39).

These results were in accordance with Epasinghe et al. (34) results, which stated that quercetin was efficient in preventing the dentin demineralization process and had the ability to promote remineralization of dentin owing to its small particle size. And it also increased the demineralized dentin’s ultimate tensile strength and elastic modulus (40). The results of Liu et al. (26) proved that the quercetin irrigant improved dentin collagen’s biomechanical characteristics and its resistance to biodegradation. It also proved that the dentin collagen irrigated by high concentrations of quercetin irrigant revealed higher resistance to collagenase degradation.

In our study, 2% concentration of CHX was chosen, as low concentrations of CHX have a bacteriostatic action, which results in the leaching of compounds of small molecular weight from bacteria. Whereas, high concentrations of CHX have bactericidal action, causing cytoplasmic precipitation and or coagulation, which is most likely brought on by protein cross-linking (41).

The results of the study stated that 2% CHX irrigant significantly decreased the microhardness of root canal dentin in the cervical, middle, and apical thirds and overall. This finding could be explained by the fact that CHX has a cationic compound’s capacity to bind anionic molecules, such as the phosphates found in the structure of the hydroxyl apatite of dentin, changing the calcium phosphorus (Ca/P) ratio (42). In addition, it was proposed that this variation in microhardness might be influenced by the application time and concentration of CHX irrigant. As using CHX in a high concentration or with a prolonged exposure time could change dentin mineral content (43, 44).

This result was in accordance with Oliveira et al. (43) results, which stated that 2% CHX irrigant decreased dentin microhardness significantly. Also with the result of Ari and Erdemir (44) which stated that the calcium and phosphorus levels as well as the microhardness of root dentin were lowered by a 15-minute CHX irrigant at concentrations of
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0.2% and 2%. Furthermore, our result was in the same line with the results of Haapasalo et al. (45) and Hasheminia et al. (46) which stated that the microhardness of root canal dentin was decreased after irrigation with CHX irrigant. This result was in disagreement with the results of Ari et al. (47), Patil and Uppin (48), Pascon et al. (49), and Prabhakar et al. (50) they proved that the microhardness of dentin was not affected by irrigation with 0.2% CHX irrigant. This is definitely owing to using lower concentration of CHX. And also, results of Aslantas et al. (21) and Massoud et al. (29) stated that using CHX irrigant for 5 minutes did not influence the dentin microhardness. These results might be attributed to the use of different irrigation period as they used CHX irrigant for 5 minutes only.

CONCLUSIONS

The microhardness of root canal dentin was increased after irrigation with 6.5% quercetin irrigant and also after irrigation with 2% quercetin irrigant, but to a lesser extent, whereas it was decreased after irrigation with 2% CHX irrigant.

REFERENCES


