EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 3531:3539, OCTOBER, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 212-07-2025 • Accept Date : 05-08-2025 • Available online: 10-10-2025 • DOI : 10.21608/edj.2025.403038.3551

EFFECT OF LASER ACTIVATION OF NATURAL HERBAL EXTRACTS AS FINAL IRRIGANTS ON THE MICROHARDNESS OF CORONAL PULP CHAMBER AND APICAL ROOT DENTIN

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ABSTRACT

Background: The current research analyzed and compared the impact of laser activation of natural herbal extract alternatives (black seed and green tea extract) as final irrigants against the conventional ethylenediaminetetraacetic acid solution (EDTA) on the microhardness of coronal pulp chamber and apical root dentin.

Material and Methods: 36 extracted human lower first premolars were chosen. Each tooth was sliced longitudinally into 2 halves (72 specimens) that were randomly categorized into three groups depending on the final irrigant; 7% black seed, 12% green tea extract, and 17% EDTA solution. Typical quantities of 3 ml of each solution were utilized for one minute and finally activated by diode laser for one minute. The microhardness values were assessed initially and following final irrigation by implementing a Vickers indentor with a weight (50 grams) and dwell period (10 seconds). The percentage decline in microhardness was displayed. The statistical data were evaluated through a one-way analysis of variance and the post hoc Tukey test. The t-test was employed for comparing the various microhardness values with a P value ($p \leq 0.05$).

Results: Following final irrigation, all the proposed irrigants considerably reduced the microhardness of the pulp chamber and apical root dentin ($P \leq 0.05$). There was a remarkable difference related to the decline of microhardness among either the black seed or the green tea and EDTA group.

Conclusions: laser activated 12% green tea or 7% black seed extract produced less decrease in the coronal pulp chamber and apical root dentin microhardness compared to 17% EDTA.

KEY WORDS: Black seed extract, Green tea extract, microhardness, natural herbal extracts

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INTRODUCTION

Endodontic treatment is indicated after the lack of dental pulp vitality. The pulpal remains and related bacteria were eliminated by root canal debridement in a chemical and mechanical way [1]. The microbial load is eradicated by using strong antimicrobial compounds like sodium hypochlorite (NaOCl) at different concentrations up to 5% [2]. Mechanical cleaning and shaping caused a smear layer to build atop the dentin surface, obstructing dentinal tubules. Calcium-chelating chemicals, such as 17% EDTA, can eliminate the smear layer by dissolution of the inorganic material [3]. The number of antibiotic strains was constantly increasing. Synthetic drugs had several side effects, encouraging researchers to develop herbal alternatives [4]. Green tea polyphenols were one of the herbal alternatives gained from the early tea plant shoots, Camellia Sinensis, which displayed outstanding chelating qualities [5]. Nigella sativa seeds and oil have been utilized for ages around the world to treat a variety of diseases. The N. sativa plant is referred to as black seed. The chemical constituents of the black seed include volatile oil, proteins, carbohydrates, fixed oil, alkaloids, saponins, certain fatty and amino acids, minerals, traces of elements, and heavy metals. Furthermore, it includes several inorganic constituents ranging in concentration between 1.79% and 3.74%, comprising calcium, potassium, phosphorus, sodium, and iron[6]. Activated irrigation was a potentially helpful approach since it increased the antibacterial and tissue-dissolving efficiency of the irrigants [7]. The diode laser is an efficient and convenient innovation applied in various clinical approaches. Its wavelengths vary between 655 and 980 nm and are appropriate for dental applications. This can be accomplished through using a thin, flexible fiber that can be easily inserted into curved and narrow canals, as well as unreachable areas inside the root canals. The diode laser can effectively minimize the bacteria counts inside the dentinal tubules up to 500 μm depth [8]. These irrigants and chelating compounds may alter the structural characteristics and calcium-to-phosphorous ratio of coronal and radicular dentin [9]. Any variations in this ratio could influence the early proportions of organic and inorganic constituents, affecting dentin's microhardness, permeability, and solubility. Variations in the mineral constitution of the outer dentin layer can subsequently impair the seal ability and adhesion of dental materials to the pulp chamber and root dentin, especially resin-based composite and sealers of root canals [10]. In addition, laser activation may alter dentin's chemical, physical, and microhardness properties [8]. However, inadequate data was available to evaluate the effect of laser activation of natural herbal extracts as a final irrigant on dentin microhardness. As a result, the current research analyzed and compared the impact of laser activation of natural herbal extracts alternatives (7 % black seed 12 % green tea extract) as a final irrigant to the conventional 17 % EDTA on the microhardness of pulp chamber and apical root dentin. The null hypothesis was that laser-activated natural herbal extract irrigant solutions would not positively impact the microhardness of the pulp chamber and apical root dentin following mechanical instrumentation.

METHODS

Study design and ethical approval document

The Research Ethics Committee (REC), Faculty of Dentistry, Sinai University, has accepted the study's protocol (approval no.: SU.REC.2024 (27 H). Before the study began, all the teeth were obtained from patients who had already gained the consent describing their approval of using their biologic samples, so informed consent to participate was obtained from all of the participants in the study. All patients should be free of any systemic diseases, and a suitable medical index is needed to assess their medical status.

Sample size calculation

The sample size was estimated employing G*Power version 3.1.9.7 based on the findings of a prior investigation [11]. A power analysis was conducted to ensure that there was enough power to perform a two-sided statistical test. By adopting an alpha level of 0.05 and a beta of 0.10, i.e., power = 90% and an effect size (d) of 0.65, the expected sample size (n) was 36, i.e., 12 specimens per group, to detect the microhardness differences among the tested groups.

Specimens' selection

For measuring the microhardness of the coronal pulp chamber and apical root dentin, a study included thirty-six freshly extracted human mandibular first premolars for orthodontic or periodontal purposes. They were maintained at 4°C in saline containing sodium azide (0.02%) and used at least a month following extraction. The study was planned to include single-rooted, caries-free lower premolars with fully developed roots, without any cracks or fractures. Cone beam CT was utilized to identify premolars with single-rooted canals. Teeth with internal resorption, undeveloped roots, calcified canals, and root canals lacking apical patency were excluded. A 2.6% NaOCl (5 ml) was applied for 1 hour to dissolve the tissue adhering to the root surfaces. The ultrasonic scaler was used to eradicate any residual tissue or calculus. The teeth were subsequently maintained in a regular saline solution (0.9%) until used [12].

Specimens' preparation

For teeth preparation, a high-speed handpiece with a round cutting tip and Endo-Z burs were used. After pulp exposure, a sterile tapered diamond bur was used to make an access cavity through the crown. For the goal of standardizing the working length, the crowns of the selected premolars were smoothed out beneath a stream of water coolant to create a point of reference. The specified length of the roots was 21 mm [13].

Each root's cementum was covered with cement, and a sealed canal system was created. The apical part of the root was covered with heated, soft adhesive and permitted to set prior to being put into a clear Plexiglas tube loaded with polyvinyl silicone[14]. K-file ISO #10 was pushed inside the root canal to confirm apical clearance up to its tip was clearly observed through the apical foramen. When a file initially emerged, the length was shortened by 1 mm to estimate the working length [12]. The working length (WL) was preset at 20 mm. The Protaper Next rotary system (PTN) (Dentsply Maillefer) had been employed for root canal mechanical preparation via a crown-down approach. This was performed using an endodontic motor (XSmart, Dentsply Maillefer, Ballaigues, Switzerland) that was customized to a 2 Ncm (torque) and 300 rpm (rotational speed) regarding the manufacturer's recommendations. The PTN rotary system X4 (40/6%) was worked as the master apical file until it reached the full W.L in a pecking action. Throughout the mechanical preparation, the root canals were constantly rinsed by a 2.5% NaOCl (5 mL) (Golden Falcon, Dubai, UAE) for 1 minute between instruments. Afterwards, distilled water (5 ml) was delivered into the canals for one minute to counteract the carryover action of NaOCl. Prior to the final irrigation protocol, each tooth was cut vertically into two halves (72 specimens), and each half was immersed in auto-polymerizing acrylic resin (Caulk/Dentsply, Milford, DE, USA). The dentin surfaces of each specimen were smoothed with gradually finer abrasive sheets to eliminate any existing surface roughness or scratching. The specimens (n=72) were then randomly distributed among three groups of 24 specimens each, based on the type of the final irrigant solution. **Group 1**: dentin surfaces of the specimens were rinsed with 3 mL of 7% black seed extract for 1 minute that is prepared at Nano Gate Company (Cairo, Egypt) by dissolving 2.1 gram of black seed extract in 30 mL distilled water to get homogenous solution. **Group 2**: dentin surfaces of the specimens were rinsed with 3 mL

of 12% green tea extract for 1 minute. 12% green tea was prepared at Nano Gate Company (Cairo, Egypt) by dissolving 3.6 gram of green tea extract in 30 mL distilled water. **Group 3:** dentin surfaces of the specimens were rinsed using 3 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Dent Wash,Dental,New York NY) solution for 1 minute. Each specimen was surrounded all around by a piece of pink wax in the form of a trough for holding the irrigant solution. Irrigation was administered utilizing a 30-gauge side-ventilated needle (NaviTip, Ultradent, UT, USA). Once the final irrigants were dispersed, the irrigation solutions were activated utilizing a diode laser for 1 minute, using a thin, flexible fiber tip (Clarso pico, elexxion AG dental laser, Singen, Germany). Subsequently, the samples were promptly rinsed with purified water (5 ml) to prevent extended exposure to chelating solutions and then blotted dry.

Microhardness assessment

Prior to the irrigation phase, the dentin microhardness of each tooth half was assessed utilizing a microhardness testing machine with a Vickers diamond indenter (Wilson hardness tester model TUKON 1102 Germany) and recorded as control values (V1). Three distinct indentations were created parallel to the border of the pulp chamber (2 mm atop the cement-enamel junction) and the root canal space (0.5 mm beyond the root canal edge), at a 100 μm depth from the pulp-dentin interface. Each indentation utilized a 50-gram (HV 0.05) weight and a dwell time (10 seconds), positioned at varying locations on the pulp chamber dentin and apical root dentin within each specimen. Following the final irrigation, new indentations were made for each sample close to the initials utilizing the same procedures as before (V2). The microhardness values were calculated as the mean of the indentations' data. For calculating the reduction in each specimen's microhardness values, the following formula has been used: V1-V2/V1×100 Where V1 denotes the control Vickers hardness number (VHN) and V2 indicates post-treatment VHN [15].

Statistical analysis

Statistical data analysis was carried out employing SPSS 23.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows. The values were reported as means and ranges. The recorded values were regularly distributed after having been assessed with the Kolmogorov-Smirnov test for normality checking. Consequently, a one-way analysis of variance (ANOVA) test was applied to compare groups and accompanied by Tukey's post hoc test for pairwise comparisons. The significance level was determined at $p \leq 0.05$.

RESULTS

Upon comparing the Vickers microhardness values (mean ± standard deviation) of coronal pulp chamber dentin and apical root dentin across the tested groups prior and subsequent to final irrigation treatment, as displayed in (Table 1). There was no statistically significant difference was observed prior to the final irrigation treatment. While subsequent to final irrigation, the coronal pulp chamber and apical root dentin microhardness decreased significantly ($P < 0.05$) between the 12% green tea or 7% black seed and 17% EDTA groups. Average percentage variations in Vickers microhardness values of coronal pulp chamber and apical root dentin following the final irrigation treatment are summarized in (Table 2). The EDTA group demonstrated the greatest mean percentage decline in the microhardness for both the coronal pulp chamber and apical root dentin, followed by the black seed and green tea groups. While, there was an insignificant difference between the 12% Green tea and 7% black seed groups ($P >0.05$). According to the mean percentage change, there was no statistically significant difference in the microhardness of the apical root dentin and the coronal pulp chamber, as indicated by the p-value ($P>0.05$) in (Table 3).

TABLE (1) Comparison between groups according to micro hardness of coronal pulp chamber and apical root dentin before and after the final irrigation treatment.

Micro hardness of coronal pulp chamber dentin	**Black seed group**	**Green tea group**	**EDTA group**	**F-test**	**p-value**
Before treatment					
Mean±SD	65.87±2.13	66.35±4.10	66.28±2.87	0.081	0.922
Range	62.8-68.4	59.9-72.8	61.7-72.33		
After treatment					
Mean±SD	48.33±3.50A	49.53±2.16A	42.92±2.27B	20.222	0.001*
Range	42.4-54.3	46.4-52.6	40.3-46.1		
Difference					
Mean±SD	17.53±3.15B	16.83±4.57B	23.36±2.65A	12.261	0.001*
Range	13.8-25.2	7.7-26	19.1-26.4		
Micro hardness of apical root dentin	**Black seed group**	**Green tea group**	**EDTA group**	**F-test**	**p-value**
Before treatment					
Mean±SD	45.46±6.80	49.82±4.99	47.10±4.86	1.839	0.175
Range	33.8-55.0	39.8-58.6	37.7-55.8		
After treatment					
Mean±SD	33.05±2.10B	37.66±3.17A	30.84±2.30C	22.133	0.001*
Range	28.9-37.5	31.9-40.7	26.7-33.1		
Difference					
Mean±SD	12.40±3.60B	12.16±3.40B	16.27±4.07A	2.409	0.011*
Range	1.6-21.2	4.8-19.1	10.3-22.8		

Using: One way Analysis of Variance test was performed for Mean±SD & Multiple comparison between groups through Post Hoc test: Tukey's test

Different capital letters indicate significant difference at (p<0.05) among means in the same row

p-value >0.05 is insignificant; *p-value <0.05 is significant

TABLE (2) Comparison between Groups according to mean percentage of change in Micro hardness of coronal pulp chamber and apical root dentin.

Mean % Change of coronal pulp chamber dentin	**Black seed group**	**Green tea group**	**EDTA group**	**F-test**	**p-value**
Mean±SD	26.63±4.66B	25.09±5.63B	35.20±3.20A	16.78	0.001*
Range	20.3-37.1	12.8-35.9	29.7-39.2		
Mean % Change of apical root dentin	**Black seed group**	**Green tea group**	**EDTA group**	**F-test**	**p-value**
Mean±SD	26.03±7.24B	23.82±6.85B	34.15±5.56A	8.178	0.013*
Range	4.5-39.1	10.8-37.4	23.8-43.6		

Using: One way Analysis of Variance test was performed for Mean±SD & Multiple comparison between groups through Post Hoc test: Tukey's test

Different capital letters indicate significant difference at (p<0.05) among means in the same row

p-value >0.05 is insignificant; *p-value <0.05 is significant

TABLE (3) Comparison between microhardness of coronal pulp chamber dentin and microhardness of apical root dentin according to mean percentage change.

Mean % Change	Microhardness of coronal pulp chamber dentin	Microhardness of apical root dentin	t-test	p-value
Black seed group				
Mean±SD	26.63±4.66	26.03±7.24	0.175	0.864
Range	20.3-37.1	4.5-39.1		
Green tea group				
Mean±SD	25.09±5.63	23.82±6.85	0.412	0.689
Range	12.8-35.9	10.8-37.4		
EDTA group				
Mean±SD	35.20±3.20	34.15±5.56	0.611	0.554
Range	29.7-39.2	23.8-43.6		

Using: t-Independent Sample t-test for Mean±SD; p-value >0.05 is insignificant; *p-value <0.05 is significant

DISCUSSION

Using root canal irrigating solutions for disinfection or smear layer elimination during mechanical preparation and instrumentation may induce structural alterations within the dentin surface, influencing its physical characteristics [10]. According to reports, there is a significant relation between the hardness and tooth mineral concentrations [16]. These irrigants can negatively affect the bonding of subsequently inserted adhesive restorations either directly by influencing the bonding procedure or indirectly by altering the structural and mechanical qualities of the bonding substrate (pulp chamber dentin). The reduction in the calcium and phosphorus ratios as well as the mechanical characteristics of dentin, such as flexural strength, modulus of elasticity, and microhardness, has been demonstrated following root canal irrigation, which in turn could minimize the micro-mechanical interlocking between adhesive resins and pulp chamber dentin [17]. There were insufficient data exploring the effect of laser activation of herbal extract solutions on dentin microhardness. Hence, the research analyzed and compared the impact of laser activation of natural herbal extracts alternatives (7% black seed and 12 % green tea extract) as a final irrigant to the conventional 17% EDTA on the microhardness of the coronal pulp chamber and the apical root dentin. The Vickers test was employed in this study due to its lesser sensitivity to the surface characteristics than other microhardness measuring techniques and its greater sensitivity to measurement inaccuracies when equal forces are exerted [18]. The dentin microhardness gets reduced as the indentations are created near the pulp [19]. In the current research Vickers dentin microhardness was evaluated by creating indentations at a 100 μm depth and 0.5 mm beyond the pulp walls in the apical root and pulp chamber dentin for standardization, employing a 50-gram weight and a dwell duration for each measurement (15 seconds). The canal apical portion is more critical for its narrower dimensions compared to the other parts and its harboring of a significant quantity of bacteria. Alongside the crucial instrumentation and irrigation of this apical portion. Moreover, numerous bacteria that have infiltrated the dentinal tubules, known as the apical "vapor lock," might substantially hinder canal debridement during positive pressure irrigation [14]. Activation strategies were developed to counteract the vapor

lock phenomena in the apical part [20]. The flushing effect and warming action of laser radiation on the irrigant solution could boost its efficiency and allow it to contact previously inaccessible areas in the root canal. Furthermore, the employment of a laser tip that was flexible and thin. This laser tip could affect the confined portion of the root canal within 1 mm of apical constriction activates the irrigation in this area, resulting in a powerful disinfection effect [21]. The results of the present research demonstrated an insignificant contrast in the mean percentage change of microhardness among the coronal pulp chamber and apical root dentin.

However, apical root dentin showed the greatest loss in microhardness compared to coronal pulp chamber dentin. These findings could be attributed to the lower mineral density of radicular dentin in comparison to pulp chamber dentin. This outcome was in line with previous research [22]. Furthermore, the results indicated that each of the examined irrigation solutions lowered the microhardness of apical root canal and coronal pulp chamber dentin. However, there was a substantial difference between 17% EDTA and 7%black seed or 12%green tea extract, while there was an insignificant difference between black seed and green tea extract. Nevertheless, green tea extract generated less reduction of microhardness in the coronal pulp chamber and apical root dentin than black seed extract. It could be related to the major component of green tea extract, the catechin, which constitutes one of the important green tea polyphenols [23]. Also, green tea polyphenols are a chelating agent [24]. Further explanation was that phenolic elements, particularly epigallocatechin-3-gallate (EGCG), could reduce dentin erosion by inhibiting MMPs, and the presence of low acidic pH may also be responsible for the lower decline in microhardness [25]. This study's findings were consistent with previous research, which found that green tea extract revealed a lower reduction in dentin microhardness [26].

Other studies revealed that the green tea extract might minimize the roughness and the wear caused by erosion of dentin, which contradicted our study [27]. One possible explanation for the discrepancy could be the different approaches used. The effect of black seed on the coronal pulp chamber and apical root dentin could be associated to its chemical constituents, which possesses caries resistant elements such as phosphorous and calcium, that are essential components of the tooth structure [28]. This could serve to improve the tooth structure's microhardness.

The results of this study were supported by other research, which concluded that the rise in the black seed extract concentration could boost the mineral concentration in the extract, leading to a higher calcium-phosphate ratio (Ca\P), thus hardening the tooth structure [29]. Furthermore, the study's findings showed that a 17% EDTA solution dramatically decreases the microhardness of the coronal pulp chamber and root dentin. By eliminating calcified dentin ingredients, chelating agents soften dentin and promote a decrease in dentin microhardness [30]. From the research's limitations, a substantial amount of the irrigant can be applied uniformly while maintaining similar with the apical root and coronal pulp chamber dentin surfaces. This is not simulating the clinical situations due to the existing of the apical vapor lock. Another limitation was the limited accessibility of the laser flexible tip application that could be encountered, reducing the proper penetration in the clinical conditions.

CONCLUSIONS

Under the present research limitations, the coronal pulp chamber and apical root dentin microhardness were decreased following laser activation of the proposed irrigation solutions. However, 12% green tea and 7% black seed extract showed the least decrease in the apical root and coronal pulp chamber dentin microhardness. So, the null hypothesis tested was rejected as the tested herbal extracts had favorably impacted the dentin

microhardness. Lastly, it is suggested that additional in vivo research be required to examine how these herbal irrigants affect the bonding properties and mechanical behavior of the coronal and radicular dentin.

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