EFFECT OF AQUEOUS ALOE VERA EXTRACT AS ROOT CANAL IRRIGANT ON ROOT FRACTURE RESISTANCE: A COMPARATIVE IN VITRO STUDY

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ABSTRACT

This study was aimed to evaluate the effect of aqueous Aloe vera on susceptibility to root fracture resistance (RFR) in human teeth subjected to endodontic preparation, and compare it with chemical irrigants (sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and SmearOFF). A total of 40 human maxillary first molars that were extracted and had a straight palatal root. These roots were distributed randomly based on the irrigation regimes (n=8): G1: Distilled water; G2: 3% NaOCl; G3: 3% NaOCl + 17% EDTA; G4: Smearoff; and G5: 90% Aloe vera, and then endodontically prepared using the ProTaper Next rotary system. The samples were placed within acrylic molds using periodontal simulation and exposed to compressive forces at a rate of 0.5 mm/min until fracture was noted. The specimens undergo axial forces on a universal testing machine during mechanical compression testing. The force necessary for fracture was measured and recorded in Newton (N). The results showed that the mean fracture resistance of Aloe vera group was the highest while NaOCl group was the lowest. It can be concluded that, the fracture resistance was significantly different in various irrigation protocols.

Keywords: Aloe vera, NaOCl, EDTA, Smearoff.

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تأثير مستخلص الصبار المائي كغسول للقنوات على مقاومة كسر الجذر: دراسة مقارنة في المختبر

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المستخلص

الهدف من هذه الدراسة هو تقييم تأثير مستخلص الصبار المائي على مقاومة كسر الجذر (RFR) في أسنان الإنسان المعرضة للتحضير اللبي، ومقارنتها مع الري الكيميائي (هيبوكلوريت الصوديوم (NaOCl)، حمض الايثلين دياminيتراسيتك (EDTA)، مزيل الطبقة اللطاخه (SmearOFF)) . مجموعه 40 من أضراس الفك العلوي البشرية التي تم قلعها ولها جذر حنكي مستقيم. تم توزيع هذه الجذور بشكل عشوائي بناء على أنظمة الري (ن =8) :م1 (الماء المقطر)؛ م2 (3٪NaOCl)؛ م3 (3٪ NaOCl+٪17 EDTA) ؛ م4 (SmearOFF) ؛ م5 (90 ٪ :صبار) ، و تم تحضيرها لبيا باستخدام نظام ProTaper Nextالدوار. وضعت العينات داخل قوالب الاكريليك باستخدام محاكاة اللثة وتعريضها لقوى الانضغاط بمعدل 0.5 مم/دقيقة حتى لوحظ الكسر. تخضع العينات لقوى محورية على آلة اختبار عالمية أثناء اختبار الضغط الميكانيكي. تم قياس القوة اللازمة للكسر بالنيوتن .أظهرت النتائج أن متوسط مقاومة الكسر لمجموعة الصبار كان الأعلى بينما كانت مجموعة NaOCl هي الأدنى. يمكن الاستنتاج أن مقاومة الكسر كانت مختلفة بشكل كبير في بروتوكولات الري المختلفة.

الكلمات المفتاحية: الصبار، هيبوكلوريت الصوديوم، حمض الايثلين دايمينيتراسيتك، مزيل طبقة اللطاخة

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INTRODUCTION

Root canal treatments aims to achieve three-dimensional obturation and completely seal the root canal system (2).The main challenges for irrigation are the complex nature of human anatomy and the presence of microorganisms in the form of surface-attached biofilm formations (16). Different bacteria have varying susceptibility to antiseptics and disinfectants (17). *Enterococcus faecalis*, a common etiological agent in failed endodontic treatments, thrives in nutrient-poor root canals and alkaline tooth environments after intracanal medication treatment. It is often found in failed endodontic therapies (36). In order to ensure long-term success, it is crucial to obtain a filled root canal that is devoid of voids and to prepare the root canal chemically and mechanically (5) . Endodontic irrigants that are frequently used include sodium hypochlorite and Ethylene Diamine Tetraacetic Acid, but the extended application of these substances at higher concentrations has detrimental impacts on the physical characteristics of root canal dentine. These consequences include a notable decrease in flexural strength, elastic modulus, and microhardness, potentially elevating the susceptibility to root fractures (45). Despite the strong effects of these compounds, their drawbacks are equally severe. The cytotoxicity of these chemical solutions has been emphasized in several investigations (11, 14, 23). The antibacterial and therapeutic properties of natural plant extracts, along with their minimum or negligible cytotoxicity, display the possibility for application as an endodontic irrigant (48). Plants have been utilized as a medicinal resource for treating disorders in various regions of the world (26).Medicinal plants play a crucial role in maintaining the health of individuals and communities worldwide (37).Herbal plants are widely recognized as significant medical plants due to their ability to effectively treat many diseases and illnesses. This is attributed to the presence of several secondary metabolites or bioactive substances in these plants (4). Aloe Vera, a member of the Liliaceae family, is utilized in traditional medicine (32). Aloe vera leaves include two distinct substances: a bitter yellowish liquid found on the outside layer immediately before the external covering, and a gel located on the inner side of the leaf. Additionally, it is employed in the management of many disorders, including those related to dermatological conditions and injuries (18, 19).Aloe vera extract may be evaluated as an oral hygiene aid to decrease the formation of plaque (1, 6). The biocompatibility of Aloe Vera is very high. Numerous researches have been conducted to investigate the potential use of this substance as an irrigant and medicament in root canal treatment due to its potent anti-inflammatory, antibacterial, and antifungal properties (47). The World Health Organization (WHO) promotes the utilization of therapeutic plants and herbs (28). No studies have been conducted to date to assess the effect of Aloe Vera on root fracture resistance (RFR). Thus, this study aimed to compare the influence of various irrigants on RFR. The null hypothesis of this study was that different irrigation solutions did not affect the fracture resistance values of roots.

MATERIALS AND METHODS

Ethical approval from The Research Ethics Committee of the College of Dentistry, University of Baghdad (Project No. 821523, Ref. No. 821) was gained for the use of extracted human teeth. Forty extracted human maxillary first molars were included in this study. Each tooth should have a Straight palatal root, and the length of Palatal root 12 mm. Roots were examined using a stereomicroscope at 40x magnification, and those with immature apices, caries, restorations, calcification, fractures, or cracks were excluded from the study. The teeth will be stored in plastic containers contain distilled water and 0.1% thymol (sigma-Aldrich, Steinheim, Germany) to avoid dehydration and to prevent bacterial growth at room temperature. To eliminate any variability in access preparation, for standardizing the length of the palatal root and for providing straight-line access during the instrumentation a digital caliper was used for measuring the length of the root and marked by a permanent marker at 12 mm from the root end to the coronal point. Palatal roots of the teeth were cutting perpendicular to the longitudinal axis of the roots using diamond disc of 0.3 mm thickness and mounted on straight handpiece at low-speed (1500 rpm) under constant copious amount of tap water. The dimensions of the roots were measured mesiodistally and buccopalatally, using a digital vernier caliper. In order to standardize the dentin thickness and to confirm the presence of unmanipulated root canal, all roots were radiographed mesiodistally and buccopalatally. The specimens presenting a difference of 20% from the mean dimension were discarded. The pulpal tissue was removed with a barbed broach and a stainless steel K-File size 10 was inserted into the root canal until the tip was seen just exiting at the apical foramen (observed under magnifying lens). The working length was determined by subtracting 1 mm from this length (11mm)(3). The diameter apically was standardized to K file # 15 and any root with an apical size larger than #15 was discarded and substituted with another specimen. The apical foramen was sealed with sticky wax to prevent extrusion of irrigants out of the canal. palatal roots were randomly divided into 5 groups according to the irrigation protocol (N=8) (3):

Group 1 (Distilled water): The irrigation was done by using distilled water in the following sequence: 2mL distilled water for 1 minute after each instrument change. Final rinse of 5mL distilled water for 1 minute.

Group 2 (3% NaOCl): The irrigation was done by using 3% NaOCl (Vista Apex, Racine, United States) in the following sequence: 2 mL 3% NaOCl for 1 minute after each instrument change, 5 mL 3% NaOCl for 1 minute. Final rinse of 5 mL distilled water for 1 minute.

Group 3 (3% NaOCl + 17% EDTA): The irrigation was done by using 3% NaOCl followed by 17% EDTA (Cerkamed, Stalwa Wola, Poland) in the following sequence: 2 mL 3% NaOCl for 1 minute after each instrument change, 5 mL 17% EDTA for 1 minute. Final rinse of 5 mL distilled water for 1 minute.

Group 4 (SmearOFF**):** is a mixture of Chlorhexidine and EDTA. The irrigation was done by using Smearoff (Vista Apex, Racine, United States) in the following sequence: 2mL Smearoff for 1 minute after each instrument change, 5 mL Smearoff for 1 minute. Final rinse of 5 mL distilled water for 1 minute.

Group 5 (Herbal product-90% Aloe vera): The irrigation was done by using 90%aloe vera in the following sequence: 2mL 90% Aloe vera for 1 minute after each instrument change, 5 mL 90% Aloe vera for 1 minute. Final rinse of 5mL distilled water for 1 minute.

Preparation of the 90%Aloe Vera Extract

Aloe vera is a highly abundant source of more than 200 bioactive substances including sugars, saponins, carotenoids, flavonoids, tannins, Anthraquinone, steroids, vitamins, minerals, enzymes, polysaccharides, alkaloids, phenolic compounds, phenols, and organic acids (18). The plant of A. vera (leaves) was harvested from a local farm in Karbala and Mature healthy of fresh green leaves of Aloe Vera were cut then thoroughly washed with sterile distilled water to remove dirt, finally we used 70% ethyl alcohol to disinfect surfaces of the leaves to be ready to extract the gel from inside. After cutting their thick epidermis was removed and the fresh semi-solid mucilaginous gel (pulp) in the center of these leaves was collected in a sterile container under aseptic environment with aid of spoon and knife. The straw coloured gel was homogenized by blender, filtered by using Millipore filter 0.45 mm. We used 10 milliliters of distilled water to dissolve 90 grams of the gel to obtain 90% Aloe Vera solution (7). Aloe Vera solution prepared immediately before instrumentation.

Root Canal Instrumentation

A smooth glide path was achieved by the utilization of ISO No.10 and No. 15 manual files. After determining working length (WL) and confirming a smooth, reproducible glide path, the canals were instrumented using the ProTaper NEXT (PTN) rotary system (Dentsply Maillefer, Ballaigues, Switzerland) up to size X3 (30.07), following the manufacturer's instructions. Endodontic side vented needles, gauge 27, were used to irrigate the canals. The needles were injected within 2 mm of the working length, and a stopwatch was used to control the irrigation time. Following irrigation techniques, paper points X3 (Dentsply Maillefer, Ballaigues, Switzerland) were used to dry every canal. A single operator carried out all of the procedures (3).

Preparation for the fracture test

Using a digital calliper, the coronal 2 mm of the root was measured and marked with a permanent marker to ensure it remained outside the mould. The instrumented root was dipped into melted wax for PDL simulation up to 2 mm below the specimen's most coronal portion, creating a layer of wax that was between 0.2 and 0.3 mm thick. The chemically cured acrylic resin was mixed in accordance with the manufacturer's instructions and poured into a specially designed plastic mould (25 mm high and 23 mm diameter). Before the acrylic resin reached the dough stage, the root with MAF in situ was dipped into the resin up to 2 mm of the specimen's coronal end. During the mounting process, a protractor was used to make sure the root's long axis was vertically aligned, ensuring that the MAF, root's and acrylic block, long axes were all coincident and perpendicular to the horizontal plane (29) (38) (39) . The specimen was submerged in cold water to prevent overheating following the initial polymerization. Once the acrylic polymerization process was finished, the root was taken out from the resin block and the wax separated from the root surface. The resin "alveolus" for the periodontal ligament simulation was filled with a polyvinyl-siloxane material (light body), the root was then placed within, and any extra material was cut away with a scalpel blade. The acrylic blocks were secured to a universal testing machine's lower plate. The machine's customized stainless steel rod tip which is rounded in shape and 4 mm in diameter, was oriented over the canal orifice and parallel to the long axis of the root. After that, the vertical force was gradually increased (by 0.5 mm/min) until the fracture happened. The testing machine recorded a quick and steep drop along the load/time curve, which confirmed the fracture moment. The force necessary for fracture was measured in Newtons (N) and for the majority of specimens, an audible crack was also detected.

STATISTICAL ANALYSIS

The data were assessed for normality using the Shapiro-Wilk test. To compare the means of the sample groups with respect to the RFR, analysis of variance (one-way ANOVA) and a subsequent multiple comparison test (Tukey Post-hoc tests) were used with a confidence level of 95%. All statistical analyses were performed using the SPSS software version 26 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The data for fracture resistance (Newton) of five groups are presented in Table (1). The mean fracture resistance of Aloe vera group was the highest while NaOCl group was the lowest. Comparing the mean fracture resistance of five groups, ANOVA showed significantly different fracture resistance among the five groups ($F=7.690$, $P<0.000$).

Table 1. Comparison of Mean Compressive Load of different Groups

Groups	Minimum	Maximum	Mean	Std. Deviation	P Value (ANOVA)
G1.D. W	703	1022	891	114.87	
G2. NAOCL	598	866	717.5	100.748	
G3. NAOCL/EDTA	657	909	743.25	100.639	
G4. SmearOOF	703	987	838.875	110.397	0.000*
G5.A. V	798	1218	996.125	145.126	

***D.W: Distilled water**
***A.V: Aloe vera**

One Way ANOVA Applied P≤0.05, *Significant

Intergroup multiple comparisons were done by Tukey Post-hoc tests, at a level of confidence interval of (95%), which as well revealed significant differences of group 1 with group 2, group2 with group5 and group 3 with group 5 as shown in Table (2).

Table 2. Tukey Post-hoc tests among groups

Groups		Mean Differences	SE	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	173.500	57.759	.037	7.438	339.561
	3	147.750	57.759	.101	-18.311	313.811
	4	52.125	57.759	.894	-113.936	218.186
	5	105.125	57.759	.379	-271.186	60.936
2	3	-25.750	57.759	.991	-191.811	140.311
	4	121.375	57.759	.242	-287.436	44.686
	5	278.625	57.759	.000	-444.686	-112.563
3	4	-95.625	57.759	.474	-261.686	70.436
	5	252.875	57.759	.001	-418.936	-86.813
4	5	157.250	57.759	.071	-323.311	8.811

***.SE: Standard error.**
***.Sig.: Significant.**
***.The mean difference is significant at the p≤ 0.05 level.**

The objective of this in vitro investigation was to assess the impact of Aloe vera and chemical irrigants on the possibility to root fracture resistance (RFR) of human teeth subjected to endodontic preparation. In this study, there was a significant variance in fracture resistance observed among various irrigation regimes. Thus, the null hypothesis was rejected. Root fractures are a dangerous side effect that can happen during or after root canal therapy and frequently result in tooth extraction (22) . Pressure during the filling procedure and tissue loss during instrumentation are risk factors that could result in root fractures in teeth that have received endodontic treatment (25) . Irrigation solutions have an impact on the fracture resistance of teeth that have received endodontic treatment, which is another important factor in root fracture (9) . In this study, the tooth's minimal fracture resistance was demonstrated by sodium hypochlorite (NaOCl), the gold standard in root canal therapy. This outcome is consistent with earlier research conducted by Khoroushi et al. (2017) (24) and Sinha et al. (2023) (41). This may be explained by the fact that NaOCl reduces dentin's hardness due to a reduction in the intertubular dentin matrix's stiffness brought on by the mineral phase's uneven distribution within the collagen matrix (21). NaOCl decomposes into sodium chloride and oxygen, oxidizing some elements of the dentin matrix and reducing the dentin's elastic modulus and flexural strength, because of the loss of organic material from the dentin (20, 33). Despite the fact that EDTA and NaOCl were frequently used for irrigation, research has shown that these chemicals negatively alter the modulus of elasticity, flexural strength, permeability, solubility of dentin and reduction in the fracture resistance of teeth that have had endodontic treatment (10, 15, 24, 31). In relation to the potential for clinical incidents, these characteristics may result in a decrease in the root canals' resistance to functional loads and an increase in the root's susceptibility to fracture. These factors can be responsible to the results from groups 2 and 3, which had the lowest values of fracture resistance. These outcomes were consistent with lots of earlier investigations (10, 15, 24, 34, 43). The aforementioned investigations have demonstrated that the control group samples, which consist of saline or distilled water, require a greater load to fracture as compared to the samples treated with usual irrigating chemicals such as NaOCl or NaOCl/EDTA. In contrast to the aforementioned findings, Marcelino et al. (2014) (27) demonstrated that the fracture resistance of the samples subjected to NaOCl treatment did not exhibit any significant differences compared to the control group treated with deionized water. This suggests that the chemical agents did not have an impact on the fracture resistance. The inconsistent outcomes could be attributed to differences in the methodology used. The current investigation demonstrated that the fracture resistance of roots irrigated with distilled water exhibited greater values compared to those treated with NaOCl/EDTA. This finding disagrees with the findings of

Turk et al. (2017) (44) , who reported that the fracture resistance of samples treated with NaOCl/EDTA was higher than that of samples treated with distilled water. The divergent outcomes seen could potentially be attributed to variations in the employed methodologies. Specifically, researchers utilized a lower concentration of NaOCl (2.5% NaOCl) and employed obturation techniques (filling root canals with gutta-percha and epoxy resin-based root canal sealer using a single-cone technique). Based on the research conducted by Beltz et al. (2003) (12) , it was shown that samples with the smear layer removed demonstrated increased fracture resistance. This phenomenon might potentially be related to the demineralizing properties of 17% EDTA, as well as its capacity to eliminate inorganic constituents present in the smear layer. By removing the smear layer, the surface energy was modified, facilitating the passage of sealers into the dentinal tubules and leading to enhanced adhesion (13). According to the results of this study, there was no statistically significant difference observed in root fracture resistance between the NaOCl group and the NaOCl-EDTA group. EDTA irrigation does not reduce the fracture resistance of roots, as confirmed by Khoroushi et al. in 2017 (24). There is a limitation of prior research about the impact of SmearOFF, a mixture of Chlorhexidine and EDTA, on the resistance of root fractures. Chlorhexidine as irrigant solution is considered advantageous due to its broad-spectrum antimicrobial properties, compatibility with tissues, and non-interaction with the dentinal organic phase, thereby preserving the properties of dentin. However, it does not possess the ability to dissolve tissues (30) (35). The fracture resistance of the SmearOFF group was higher than that of the NaOCl and NaOCl-EDTA groups may be due to preservation of dentin characteristics by chlorhexidine and the deleterious effects of sodium hypochlorite on it. According to the findings of Uzunoglu et al. (2016) (46), it was observed that specimens irrigated with a QMix consisting of EDTA, CHX, and a detergent exhibited greater resistance to vertical root fracture in comparison to specimens irrigated only with EDTA.Herbal substances have been utilized by endodontists since earlier times as a means prevent the cytotoxic impact of commonly employed irrigants and to effectively eradicate bacteria that are specifically present within the dentinal tubules of the root canal system. The medical field has getting focused the utilization of natural plant extracts (42). Inadequate literature is available that depicts role of irrigation with herbal extracts (Aloe Vera) on fracture resistance of the teeth, so the present study was conducted to know the effect of Aloe Vera irrigants on the resistance to fracture of roots. Interestingly, the force necessary for fracture in the group treated with the Herbal product (aloe vera) was greater in comparison to the control group. The findings of this investigation are consistent with the prior research conducted by Sinha et al. (2023) (41), which shown that herbal irrigants, can serve as viable substitutes for NaOCl due to their significant fracture resistance, as observed in the present study. The Aloe vera group exhibits the highest level of fracture resistance, which can be attributed to the presence of calcium, one of its active constituents. According to Silva *et al*. (2016) (40), the remineralizing power of a 90% Aloe vera solution may be attributed to the deposition of calcium on the surface of dentin. The mineralization of dentin can influence its hardness attribute (J A, JJ ten B, 1992) (8). Aloe vera has been found to enhance the surface microhardness of the dentin wall in relation to the root. The in vitro environment limits the investigation. Consequently, it is not feasible to extrapolate the findings to clinical scenarios. Agitation devices can influence the effectiveness of irrigants. Furthermore, that the stress that was applied was static, which does not accurately reflect the dynamic load experienced in the oral cavity. Therefore, the findings of this laboratory experiment may not accurately represent real-world conditions. Dentists should recognize that root fractures are complicated problems that are greatly affected by a patient's unique clinical condition. Hence, more investigation is necessary to explain the influence of alternative irrigation methods that differ in terms of the duration and concentration of irrigants employed. Additionally, it is crucial to determine the consequences on dentin tissue and their association with root fracture.

Within the limitations of this in-vitro study, the findings indicate that the application of root canal irrigation had an impact on the root fracture resistance. Concerning irrigation solutions, roots that were irrigated with 90% Aloe vera demonstrated enhanced root canal strength following preparation and shown the greatest resistance to fracture. Herbal irrigants possess several advantages in terms of accessibility, cost-effectiveness, and less adverse effects in comparison to conventional alternatives, while also demonstrating enhanced fracture resistance. Additional research is required to examine the impact of various irrigants on root fracture resistance.

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