Original Article

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Dentistry Section

Comparative Evaluation of the Effect of Herbal Irrigants on the Push-out Bond Strength of Epoxy Resin Based Sealer to Root Dentin: An In-vitro Study

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ABSTRACT

Introduction: Favourable outcome of root canal treated teeth depends on the irrigants used. Sodium Hypochlorite (NaOCl) is one of the most commonly used irrigants in endodontic treatment. NaOCl has cytotoxic effects; limited ability to eliminate bacteria, along with jeopardizing of the bond strength between epoxy resin-based sealers and root dentin. Contemporary medicine orchestrates the use of biological medical extract from natural plants. Herbal alternatives are coveted as they are readily available, less expensive, have enhanced shelf life and low toxicity. Despite that, literature is sparse regarding the effect of herbal irrigants on Push-out Bond Strength (PBS) of sealer to root dentin.

Aim: To compare and assess the effect of the PBS of epoxy resin based root canal sealer to root dentin following the use of two novel herbal irrigants,10% proanthocyanidins (PAs) and 2% *Azadirachta indica* (neem extract) with 3% NaOCl.

Materials and Methods: This in-vitro study was conducted in the Department of Conservative and Endodontics at KLE VK Institute of Dental Sciences, Belagavi, Karnataka, India, between June 2019 and August 2019. The study was done on 30 extracted single rooted human mandibular premolar teeth, of which root canals were prepared using rotary instruments. The use of standard susceptibility tests {Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)} was done to determine appropriate concentration of the irrigant against *E. faecalis*. The teeth samples were randomly divided into 3 groups (n=10 each). Group 1 has teeth irrigated with 3% NaOCl (control group), Group 2 has teeth irrigated with 10% grape seed extract (10% proanthocyanidins) and Group 3 has teeth irrigated with 2% neem leaf extract (2% *Azadirachta indica*). Obturation of all the three groups were done with F3 gutta percha and AH Plus sealer followed by PBS testing using universal testing machine. One way Analysis of Variance (ANOVA) and Tukey's Post-hoc tests were used for statistically analysing the data. The p-value <0.05 was considered as statistically significant.

Results: Group 2 had significantly higher push-out bond strength as compared to Group 3 (p-value <0.001). However, there was no significant difference seen between Group 2 and Group 1 (p-value=0.207).

Conclusion: It was concluded that the PBS of 10% proanthocyanidins was the highest, followed by 3% NaOCl and *Azadirachta indica*. However, biocompatibility and safety of these novel intracanal irrigants has to be assessed with the help of further clinical and in-vitro experiments to validate these findings.

Keywords: *Azadirachta indica*, Irrigation, Proanthocyanidins, Root canal, Sodium hypochlorite

INTRODUCTION

Endodontic infections are caused due to various types of microbes however, they are dominated by obligate anaerobic bacteria [1]. Amongst the others, the *Enterococcus faecalis* is considered as the most persistent organisms that plays a pivotal part for the initiation of periradicular lesions. It can even survive in restricted conditions, due to physicochemical and biofilm forming characteristics of the organism that helps it to modify the prevailing nutritional and environmental conditions [1]. Endodontic treatment success depends on a 3-dimensional obturation of the root canal space with adhesion between root dentin, core material and sealer [2].

The type of instruments and irrigants used during instrumentation plays a pivotal role in neutralisation and inactivation of bacterial toxin creating a positive influence on the healing activity post endodontic treatment [2]. An ideal root canal irrigant is the one which has good antimicrobial action, is non cytotoxic, and does not cause any alteration of dental microstructure [2]. Sodium Hypochlorite (NaOCl) (0.5-6%) is a unique and incomparable irrigant in various endodontic treatment procedures, which not only abolishes microorganisms but also disintegrate and denature proteins. It is comparatively cheaper and has a good shelf life [3]. One of the drawbacks of this irrigant is that it impedes with the polymerisation of bonding resin of the root canal sealers due to the availability of residual oxygen present in the dentinal tubules post irrigation, which decreases of the bond strength between resin obturating materials and root canal dentin [4].

Herbal extracts are more biofriendly and suited for endodontic irrigation due to their antimicrobial, antioxidant, anti-inflammatory, sedative and anxiolytic properties there by considered as ideal endodontic disinfectants [2]. Proanthocyanidins (grape seed) are a complex subgroup of the flavanoid compound, found to be having good antibacterial, anti-inflammatory, antiallergic as well as vasodilatory properties. Apart from this, Nuttall SL et al., in 1998 has proven that Proanthocyanidins exhibit good antioxidant properties and inhibit collagen breakdown [5-8]. Its ability of eliminating/removing the free radicals have been clearly reported in literature which is shown to be more effective against the residual oxygen.

Literature has shown wide spectrum antioxidant and antimicrobial activities of neem, ginger, garlic, aloe vera and turmeric amongst which neem extract has been proven to be an immensely versatile medicinal plant used in dentistry which makes it an excellent future endodontic irrigant [9-11]. These properties help in eliminating/eradicating the residual oxygen layer formed on the root dentin, allowing better tubular penetration of sealer and enhanced polymerization leading to a 3-dimensional fluid tight seal between root dentin, sealer and gutta percha (core material). This may provide a better seal of the root canal space [12].

The other properties of herbals such as antimicrobial and anti-inflammatory have been proved but there is a lacuna in the interaction of these herbal irrigants on root dentin and its effect on the push-out bond strength. Hence, the present study was done to evaluate the effect of 2% *Azadirachta indica* (neem leaf) and 10% proanthocyanidins (grape seed) irrigating solutions on the PBS of epoxy resin based root canal sealer to root dentin.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Conservative and Endodontics at KLE VK Institute of Dental Sciences, Belagavi, Karnataka, India, between June 2019 and August 2019. Institutional Ethics Committee approval was taken for the study (number IEC/VKIDS/216).

Inclusion criteria: Extracted human single rooted single canal mandibular premolar teeth with patent canals, teeth with apical width corresponding to No. 20 K-file or less were included in the study.

Exclusion criteria: Teeth with carious lesions, apical width more than No. 20 K-file size, with calcified canals and teeth with fracture/crack or a restoration were excluded from the study. Also teeth with anatomic variations such as extra canals, curved canals and C-shaped canals were also excluded from the study.

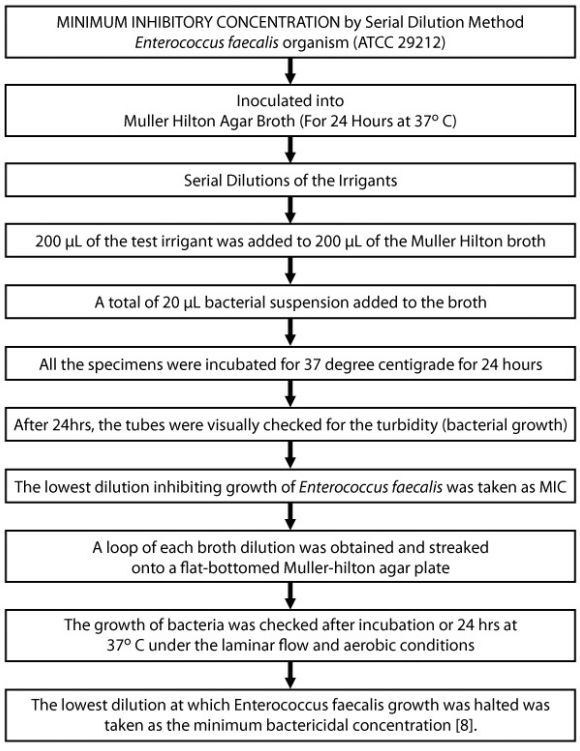
Procedure

Total 30 human permanent, intact and mature premolar teeth with single canals, recently extracted for periodontal reasons were selected by direct clinical examination for this study. They were divided into three groups of n=10 each as per the following:

- **Group 1-** NaOCl as the irrigant,
- **Group 2-** Grape seed extract as the irrigant,
- **Group 3-** Neem leaf extract as the irrigant.

The specimens were cleaned using ultrasonic scaler and distilled water followed by immersion in 0.1% Thymol solution (manufacturer: SD Fine Chemicals Limited, Mumbai) for disinfection until use [13].

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of Irrigants: The use of standard susceptibility tests (MIC and MBC) was done on *E. faecalis* (ATCC 29212) organism to determine appropriate concentration of the irrigant to be taken [Table/Fig-1].



[Table/Fig-1]: Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of irrigants.

Preparation of Irrigants

Grape extract irrigant- 10% solution; Neem extract-100 mL distilled water with 2 mg neem extract (2% extract).

The above concentrations were based on the results derived from MIC and MBC.

Preparation of 2% pure neem extract: The fresh neem leaves were procured from KLE University Shri BM Kankanwadi Ayurvedic Mahavidyalaya. The leaves were dried and powdered. Total 25 gms of neem leaf powder was measured in sterile disposable cup to which 50 mL of absolute ethanol (SD Fine Chemicals Limited, Mumbai) was added. Maceration of this mixture was done for 1-2 minutes and the extract obtained was filtered using filter cloth (Muslin) and Cellulose filter paper (Whatman). The coarse residue obtained was again subjected to the same process using 25 mL of ethanol. The extracts obtained in the above two steps were mixed together and filtered. The alcohol content in the extracts was removed by allowing the extract to boil on water bath till the volume was about 25 mL. The prepared extract was stored in an airtight sterile plastic container at 2-8°C. The final product was collected and stored for further test [10]. A 100 mL distilled water was mixed with 2 mg neem extract to obtain 2% pure neem extract solution.

Preparation of 10% grape seed solution (stock solution): The grape seed extract (PAs) (95% i.e., 95 mg per 100 mg) was procured from Bulk Supplements (Alpspure Lifesciences Private Limited, Delhi, India). The stock solution of the extract was prepared direct by taking 10 mg of the grape seed extract to which 100 mL of distilled water was added thus producing a concentration of 10%.

Preparation of the Sample

Decoronation of the teeth was done to obtain a standardised tooth length of 12 mm followed by introduction of (MANI INC, JAPAN) for determining the true length of each root until its tip appeared through the major apical foramen. The canal patency was maintained and checked with a #10 K file. Followed by subtraction of 1 mm from the actual length which helped in determination of the working length modeling wax No.2- the Hindustan Dental Product (HDP) to prevent extrusion of irrigating solutions. All the canals were prepared by a postgraduate student in Conservative Dentistry and Endodontics Department. After standardising the root length to 11 mm, the canals were prepared in the following sequential manner Propter Sx, S1, S2, F1, F2, F3 along with the irrigants using NiTi rotary files (Protaper Universal, Dentsply, India); Apical gauging and manufacturer's instructions were followed and the master apical file (F3=0.30 mm) was used based on the apical gauging.

During the preparation of the canal, a total of 5 mL of 3% NaOCl (Manufacturer-Vishal Dentocare, Ahmedabad, Gujarat) was used for irrigation between instruments. After instrumentation all the root canals were rinsed with 5 mL of 17% Ethylenediaminetetraacetic Acid (EDTA) (Manufacturer- Canalarge, Ammdent, Punjab, India). It was done to remove the smear layer.

The final flush of the canals was done with their respective solutions, i.e., Group 1- NaOCl, Group 2- Grape seed extract, Group 3- Neem leaf extract. Keeping a standardised quantity and contact time of 5 mL and 5 minutes, respectively. Lastly the root canals were additionally rinsed with 5 mL of distilled water and the canals of all the samples were dried with paper points (Diadent Group International, Korea).

The canals were coated with AH Plus sealer (Dentsply, De Trey, Konstans, Germany) with the aid of a lentulospiral (Mani Inc., Tochigi, Japan) and obturated (single cone obturation technique) using F3 gutta-percha (DentsplyDeTrey, Konstans, Germany). The root samples were coronally sealed with intermediate restorative material (3-4 mm in

thickness) (IRM, Dentsply DeTrey, Konstans, Germany). The samples were kept in 100% humidifier for 1 week.

Push-out Bond Strength (PBS) Testing

A cylindrical mold was filled with auto polymerising acrylic resin (Manufacturer-Astra Chemtech Private Limited, Mumbai, India) and the roots were vertically embedded in it, until the acrylic resin set. Acrylic blocks were removed from the mold, and 1.5 mm thick slice was obtained from coronal, middle, and apical third of each embedded root sample using a rotating diamond disk under water-cooling. The middle segment was taken for the study. Each slice was subjected to PBS test in a universal testing machine (Tecsol, Chennai, India) using a metallic indenter with a round cross-section and diameters of 0.5 mm customised to test slices. A cross-head speed of 1 mm/min was used [7].

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software (IBM SPSS statistic for windows, version 20.0. Armonk, NY: IBM Corp). Data was entered as mean, standard deviation and standard error of mean. Statistical analysis was done using one way Analysis of Variance (ANOVA) and Tukey's Post-hoc test. The p-value <0.05 was considered as statistically significant.

Null hypothesis: There was no statistical difference in the push-out bond strength of the three intracanal irrigants used.

RESULTS

The following order of PBS was observed in different groups in descending order: group 2> group 1> group 3 [Table/Fig-2].

There was a significant difference between the mean values of push-out bond strengths of the three groups (p-value <0.001) [Table/Fig-3].

S. No.	Group 1 (N=10)	Group 2 (N=10)	Group 3 (N=10)
1	9.9	11.7	5.85
2	7.7	11.7	7.8
3	8.75	7.8	7.8
4	9.67	11.7	5.85
5	9.67	7.8	3.9
6	7.8	9.75	5.85
7	7.85	7.8	7.8
8	9.75	11.7	5.85
9	8.87	11.7	9.75
10	9.75	9.75	5.85
Mean	8.97	10.14	6.63
SD	0.9041	1.7919	1.6444
SE	0.2859	0.5667	0.52

[Table/Fig-2]: Push-out bond strength values (in Newton), mean, standard deviation and standard error of the three irrigants.

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	2	63.8898	31.9449	14.2349	**0.001**
Within groups	28	60.5912	2.2441		
Total	30	124.481			

[Table/Fig-3]: Comparison of three irrigants (NaOCl, Grape seed, Neem leaf) with mean Push-out bond strength by one-way ANOVA.
p-value <0.05 was considered as statistically significant

Group 2 had significantly higher push out bond strength as compared to Group 3 (p-value <0.001). However, there was no significant difference seen between Group 3 and Group 1 (p-value=0.207) [Table/Fig-4]. On the basis of the results obtained in the study the null hypothesis was rejected.

Groups	Q statistic	p-value	Inference
NaOCl vs Grape seed	2.4677	0.2074171	p=0.207
NaOCl vs Neem leaf	4.9417	0.0045631	**p<0.001**
Grape seed vs Neem leaf	7.4094	0.0010053	**p<0.001**

[Table/Fig-4]: Comparison of three irrigants (NaOCl, Grape seed, Neem leaf) with Tukeys Post-hoc test.
p-value <0.05 was considered as statistically significant

DISCUSSION

One of the main target of endodontic treatment is to attain a monoblock effect which would prevent the entry or abidance of microorganisms [1,14-16].

The endodontic irrigants used may cause structural and chemical alteration in the dentinal composition, thereby impacting its penetrability and affect the materials adhesion to the dentinal surface [12]. The commonly used root canal irrigants is NaOCl which have shown to have deleterious effect on the root dentin by causing degradation of root dentin collagen [13].

Also, residuum of this strong oxidizing agent or its oxidative by products are revealed to impede the adhesion of epoxy resins to the canal and the free radical polymerization of methacrylate resins which may inturn reduce the bond strength [7]. Hence, herbal irrigants may be used during instrumentation owing to their antioxidant property in addition to being an antibacterial and anti-inflammatory agent [17]. The antioxidant property may lead to scavenging of the oxidative byproducts thereby increasing the adhesion of sealer to the root dentin [17]. Previous studies have shown that they may cause inhibition of collagen breakdown along with stiffening and cross-linking of the collagen [7,17,18].

AH plus sealer was used in the current study which has been effectively proved to have the highest root dentin bond strength, the explanation for this is because AH plus sealer is an epoxy resin-based sealer which strongly forms covalent bonds with root dentin due to the opening of its epoxide ring. It undergoes homogenous polymerisation with greater bond strength when compared to the bond strength of monoblock due to the absence of photo polymerisation system as its chemical property [19].

Various tests used to measure bond strength are microtensile strength testing, shear strength testing, and push-out strength testing [20]. The method of testing the bond strength chosen for this study was push-out design. A study by Goracci C et al., concluded that push-out technique was more reliable and precise than the microtensile technique for measurement of bond strength to dentin [21]. Push-out bond strength is the measurement of resistance to dislodging force by root filling materials during function, which is a highly desirable quality [22]. The main foreseeable reason to select this test is due to the easy alignment of the samples for testing, as it is comparatively less sensitive to changes in small variations among specimens and also to the variation of stress distribution during load application [20].

Middle third of the root was chosen in this study for the push-out test as according to Üstün Y et al., the coronal and middle third part have anatomically larger root canal space when compared to the apical third which would lead to better circulation of root canal irrigants [23].

The results of the present study showed that proanthocyanidins had highest push-out bond strength followed by NaOCl and *Azadirachta indica* (neem). However, there was no significant difference seen in the push-out bond strength of proanthocyanidin and NaOCl (p-value=0.207). The push-out bond strength value of proanthocyanidin was significantly higher compared to neem leaf (p-value <0.001). The results are supported by previous studies by Nikaido T et al., and Morris MD et al., which stated that use of NaOCl during instrumentation removes the organic debris from the canal, in due course it also causes degradation of root dentin collagen and decreased the bond strength of AH Plus to dentin [24,25].

A chemical bond is seen between the epoxy resin based sealer and the amino groups of the dentin in which, collagen plays a pivotal role in the adhesion between the sealer and the root dentin [7]. According to Kumar PS et al, chloramines and protein-derived radical intermediates are dissociated from NaOCl leading to adverse effects on the pyridinoline cross-links present within the type 1 collagen [7]. Thus, irrigation with NaOCl results in a structurally compromised collagen in the root dentin [7].

Irrigation with 10% grape seed extract (proanthocyanidins) showed better push-out bond strength as compared to use of 3% NaOCl in this study, this may be due to polyphenolic structures of proanthocyanidins that are capable of forming stable hydrogen bond structures and produce non biodegradable collagen matrices [26].

Previous study by Kumar PS et al., has concluded that 6% proanthocyanidins and 25% bamboo salt had consequently increased leading to enhanced resistance to biodegradation which was due to the intermolecular and intramolecular cross-links in collagen. Hence the bond strength values of specimens treated with proanthocyanidins and bamboo salt was significantly higher compared to NaOCl (p-value <0.05) [7].

The cross-linking and stiffening of collagen and reduction in collagen degradation after treatment with proanthocyanidins along with high affinity of proline rich proteins in the collagen of proanthocyanidins might be the reason for greater bond strength in proanthocyanidins-treated specimens [26,27].

Limitation(s)

Brownish discolouration of the tooth was observed after the specimens where irrigated with proanthocyanidins stock solution. However, this warrants further studies with bigger sample size. More in-vitro studies and clinical trials are needed to evaluate the biocompatibility and safety of these novel intracanal irrigants.

CONCLUSION(S)

A 10% proanthocyanidins showed highest push-out bond strength among the three irrigants used this study, followed by 3% NaOCl and the least push-out bond strength was seen with 2% neem extract. Further long-term clinical and in-vitro studies using the similar irrigation protocol and sealers are warranted to substantiate these findings.

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