ORIGINAL ARTICLE

COMPARISON OF AN EXPERIMENTAL ROOT CANAL IRRIGANT (*SAPINDUS MUKOROSSI*) AND ETHYLENEDIAMINETETRAACETIC ACID ON MICROHARDNESS OF HUMAN DENTIN

Asad Farooq[1], Fazal ur Rahman Qazi[2], Juveria Siddiqui[3], Syed Abul Faraz[4], Abdur Rasheed[5]

[1,4] Liaquat College of Medicine & Dentistry Karachi, [2,5] Dow University of Health Sciences Karachi, [3] University of Karachi-Pakistan

Background: The treatment of infected root canals is one of the most essential areas in dentistry. The endodontic therapy comprises of a sequence of steps that includes the purging of disease and infectious tissues from the root canal system. The objective of the study is to compare the effect of an experimental herbal root canal irrigant (*Sapindus mukorossi*) and Ethylenediaminetetraacetic acid, on the micro hardness of human dentin. **Methods:** Ninety single rooted teeth were collected displayed intact external morphology the selected teeth were than divided randomly into 3 groups (n=30); 1 control and 2 experimental groups: Group A consisted of specimen treated with Ethanolic extract of *Sapindus mukorossi*. Group B consisted of specimen treated with 17% Ethylenediaminetetraacetic acid and Group C (control group) consisted of specimen of roots were sectioned immersed in distilled water. After the removal of crowns, the roots were sectioned embedded in the polymer resin leaving the root dentine exposed, the micro hardness was determined. Then, the samples from each group were treated with respective irrigant solution for 15 minutes and then observation regarding the micro hardness were noted again by using Vickers micro hardness tester. Analysis of data were obtained by application of Wilcoxon signed rank test. **Results:** The study revealed that there was no significant difference noted in dentin microhardness of group "A" sample after dipping in experimental irrigant solution (*Sapindus mukorossi*). However, the study found significant difference in context of decrease in dentin microhardness after dipping a sample in a group "B" irrigant solution (17% Ethylenediaminetetraacetic acid). **Conclusion:** In order to preserve the strength of dentin that will ultimately be beneficial for increase in survival of teeth in function, probably the herbal extract of *Sapindus mukorossi* would be better candidate for future endeavour and presented as an economic and effective alternative amongst root canal irrigants.

Keywords: Root Canal Irrigants; Ethylenediaminetetraacetic Acid; *Sapindus mukorossi*; Dentin; Microhardness

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INTRODUCTION

The treatment of infected root canals is one of the most essential areas in dentistry. The endodontic therapy comprises of a sequence of steps that includes the purging of disease and infectious tissues from the root canal system. The goal of endodontic treatment is the attainment of hermetic seal along the entire root canal system.[1–3] Following these objectives instrument introduces for sculpting the root canal, as a result a smear layer produces and the next important step must be its irrigation to wash away the debris as well as scraps the dentinal wall. Irrigant should display antimicrobial properties, able to disintegrate organic tissue and smear layer, gentle to periapical tissue and provides smoothness with no significant decrease in dentin microhardness.[4]

Various irrigant solutions are available. However, it is a frequent observation that the irrigant used in routine, such as ethylene di amine tetra acetic acid has the propensity to dentinal surface along with the removal of smear layer.[5] In reference to the effect of canal irrigant ethylenedi aminetetraaceticacid (EDTA) explained to decrease dentine microhardness [6,7] by removing calcium ions, that will probably have negative impact on structural and chemical composition, may become vulnerable to root fracture. In this perspective to preserve the tooth's strength, inorganic component (Calcium-Phosphate) ratio must be maintained and (EDTA) shows no notable antibacterial properties.[8,9] Therefore various investigators in pursuit to find alternate irrigant solution to overcome the shortcomings of

conventional irrigants and capable of providing better results in endodontics.

Sapindus mukorossi is a well-established name in term for treatment for various diseases. Its husk has the use as an expectorant and surfactant[10] its fruit are important in the treatment of numerous skin disease like eczema and psoriasis[11] its seeds have been found useful to treat oral and gastrointestinal problems and root to treat gout and rheumatic disease.[12] The major compound isolated from *Sapindus mukorossi* are Triterpenoidal saponins, exhibit antimicrobial, molluscidal, insecticidal, piscicidal and fungicidal properties can be used in the management of smear layer.[6,7]

The use of herbal root canal irrigant "Ethanolic extract of Sapindus mukorossi" has not been well thought out in the dental care especially in Pakistan, which may be the best possible candidate for future perspective that possibly offers least alteration in the mineral content. Therefore, in this context it is justifiable to test the efficacy of Herbal extract of Sapindusmukorossi in order to maintain the micro hardness (strength) of dentine ultimately helps to keep the tooth's integrity and longitivity.

The presented study aimed to compare the irrigation effect on dentine micro hardness using 17% EDTA solution and *Sapindus Mukorossi* will provide insightful understanding in quest to find such an irrigant having immense capability (properties) following preserved dentin micro hardness.

MATERIAL AND METHODS

It was an in-vitro, experimental group comparative study. Institutional review board committee of Dow University of Health Sciences, Karachi IRB approval # 2016/221, approved this study. Ninety teeth were collected in time of 6 months from March to September 2017 from oral and maxillofacial surgery department of Dr Ishratul Ebad Khan Institute of Oral Health Sciences, Karachi. Single rooted teeth displayed intact external morphology were used. The selected teeth were than divided indiscriminately into 3 groups (n=30); 1 control and 2 experimental groups: Group A consisted of specimen treated with Ethanolic extract of *Sapindus mukorossi*. Group B specimen treated with 17% Ethylenediaminetetraacetic acid (EDTA) and Group C (control group) consisted of sample immersed in distilled water.

After removal of crowns of the teeth at the cemento-enamel junction ~~area~~ using high-speed hand piece supplemented with water irrigation, the roots were vertically sectioned with the aid of diamond disc to yield two halves for each tooth, after sectioning they were embedded in the polymer resin as shown in figure 1a, ensure to leave the root dentine exposed. The specimen was than polished with an abrasive (fine category) sand paper discs to eliminate any superficial scratches.[13,14] The micro hardness was determined using Vickers micro hardness tester (HMV-G 21DT, Shimatzu Corporation, Japan) and their average value were recorded.

All the indentations were made with 300 grams and 10 seconds dwell time. At the beginning prior to dipping the samples in the categorized irrigant solutions, observations of micro hardness values were recorded.[13,14] as displayed in figure-1c. Than the categorized samples in group (A, B, C) were treated with respective irrigant solution for 15 minutes, in which they were fully immersed and then observation regarding the microhardness were noted again.[15] These tests were performed in Metallurgical Engineering Department of NED University, Karachi.

The NCSS PASS V.11 software was used for the purpose of sample size calculation. Using three groups, with 95% confidence interval and 80% power of the test, the calculated sample size was 30 teeth per group. Statistical analysis was performed using SPSS V.22.0. Micro hardness of human dentine before and after dipping in irrigant solution was compared through Wilcoxon signed rank test. Furthermore, Mann-Whitney Test was also performed between the group's comparison. *p*-value of less than 0.05 was taken as statistically significant.

RESULTS

The comparison of micro hardness of human dentine before and after dipping in categorized irrigant solutions were showed in Table-1 using Vickers microhardness tester (Wolpert Wilson 450SVA) shown in Figure-1b. Before and after mean and standard deviations of the hardness were also reported.

The study found there was no significant difference for group "A" between hardness of dentin before and after dipping in the irrigant solution (*p* value=0.342) as shown in Table-1. However, for group "B" (17% Ethylenediaminetetraacetic acid) we found the statistically significant difference between before and after treated with the irrigant solution (*p* value =0.001).

The result showed that in group "C" (control group) the mean hardness of teeth before and after dipping observed no significant difference (*p* value = 0.881) found as shown in Table-1. Hence, least reduction in hardness was found in group A. In order to strengthen our findings, we also performed between the group's comparison using Mann-Whitney Test in Table-2. After irrigation mean hardness of Group A was also significantly, differ from Groups B and C.

Figure-1a: Mounted sample on acrylic blocks for microhardness testing

Figure-1b: Vickers microhardness testing machine for dentin microhardness analysis

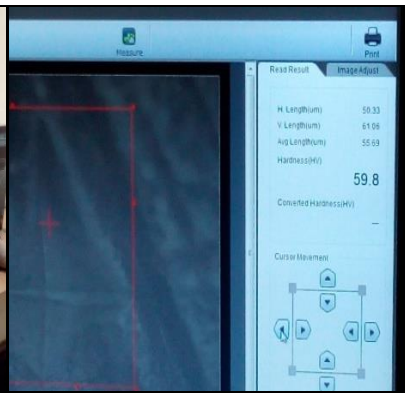

Figure-1c: Observation of dentin microhardness reading

Table-1: Before and after irrigation the micro hardness comparison for three groups.

Group A *Sapindus mukorossi*			Group B 17% EDTA			Group C Distilled Water		
	Mean (SD)	***p*-value**		**Mean (SD)**	***p*-value**		**Mean (SD)**	***p*-value**
Before	60.14(0.63)		Before	60.26(0.74)		Before	60.46 (0.40)	
After	60.07(0.49)	0.34	After	56.62(0.72)	< 0.0001*	After	60.45 (0.35)	0.88

**p*-value < 0.05 was considered as significant (Wilcoxon Signed Rank).

Table-2: After irrigation micro hardness comparison between three groups.

	Mean (SD)	***p*-value**		**Mean (SD)**	***p*-value**		**Mean (SD)**	***p*-value**
Group A	60.07(0.49)	<0.001*	Group A	60.07(0.49)	0.002*	Group B	56.62(0.72	<0.001*
Group B	56.62(0.72		Group C	60.45 (0.35)		Group C	60.45 (0.35)	

**p*-value < 0.05 was considered as significant (Mann-Whitney).

DISCUSSION

Every endodontic treatment comprises of diagnosis, treatment and the information provided to the patient. This was then followed by preparation of access cavity, identification of canal, instrumentation, irrigation and disinfection, root filling and finally coronal restoration.[16,17]

In the field of endodontics, the treatment focuses mainly on instrumentation where its main objective includes removal of content of root canal followed by proper removal of bacteria and then finally to make the process of root filling to be done as easily as possible and the ultimate achievement of root canal treatment is to preserve the tooth in functional state/position in the oral cavity.[18]

By the introduction of instruments in the canal produces a layer composed of organic as well as in organic content called smear layer [19] which must be removed before filling of root canal for proper adaptation of root canal filling material. In order to achieve, irrigant solution must be able to gain an access to canal system. Unfortunately, no irrigation solution carries the capability of acting simultaneously on both the organic as well as inorganic content of smear layer.[20]

Traditionally, the alternate irrigation by sodium hypochlorite and ethylene di amine tetra acetic acid (EDTA) is the most common practice and it has been noted by investigators that EDTA has not exhibit antibacterial activity[8,9] and—to decrease micro hardness of dentine[21] noted to facilitate inorganic component decalcification. Unnikrishnan M *et al*[22] revealed, application of 17% EDTA beyond one minute timing may results in dentinal erosion[23] which may result in vertical root fracture. Another study conducted by Nogueira BM *et al* revealed significant mineral loss using combination of 2.5% NaOCl + 17% EDTA + 2.5% NaOCl solution as a root canal irrigant[17]. Abbas FS *et al*[24] found maximum reduction in dentin micro hardness by using 17% EDTA among tested irrigants. It has been shown that NaOCl has toxic effect on vital tissues resulting in haemolysis and necrosis.[25]

Saha SG *et al*[26] found that 17% EDTA and 0.2% Chitosan, significantly decreased the micro hardness of root dentin. Chhabra N *et al*[27] has tested the canal irrigation effects by combining the extracts

of Citrus aurantifolia and *Sapindus mukorossi* in 1:1 ratio supplemented by ultrasonic agitation compared with 17% EDTA solution and revealed no significant difference between these tested irrigants in respect to removal of smear layer. Khallaf ME *et al*[28] have compared Moringaoleifera with conventional root canal irrigants (sodium hypochlorite) and found higher dentin microhardness when use in combination with chlorhexidine

Aneja KR *et al*[29] on his study depicted that *Sapindus mukorossi* displayed efficient anti-fungal properties against Saccharomyces cerevisiae. George B *et al*[30] formulated an ethanolic extract of *Sapindus mukorossi* that displayed noticeable antibacterial activity against *E. coli*, *Staphylococcus aureus* and against *Aspergillus fumigates* and *Aspergillus niger*.

Singh R *et al*[31] has tested the antimicrobial potential and found promising results against various bacteria especially against *S. typhimurium* and *E. aerogens*. Fayyad D *et al*[32] compared the effect of irrigants on dentin microhardness by release of Calcium ion (Ca) from root dentin found EDTA, QMix, and Chitosan recorded the highest Ca ion loss that ultimately leads to lower the microhardness compared to NaOCl (used as control in our study).

Our study revealed that the experimental irrigant (ethanolic extract of *Sapindus mukorossi*) showed no significant decrease in dentin micro hardness in comparison to 17% EDTA which has reduced the dentin micro hardness due to the loss of mineral content. These findings were consistent with the study conducted by Cruz-Filho AM *et al*[33] and Baldasso FE *et al*[34] who compared the reduction of microhardness among 17% EDTA, QMiX, 10% citric acid, 1% Peracetic acid and NaOCl having 2.5% concentration and revealed QMiX, 17% EDTA displayed greater reduction in dentin microhardness as compared to other tested irrigants, Eldeniz AU *et al*[35] found EDTA displayed statically similar effect in reduction of dentin microhardness compared to citric acid. So, in this context for evaluation mineral content of dental hard tissue has been carried out by measurement of dentin microhardness, in presented study "Vickers hardness test" for evaluating the surface change of dentin found suitable and adopted. Mineral loss should be minimal to maintain the structural integrity of dentin that helps to keep promising treatment outcome. Further studies must be carried out (needed) to test the efficacy against common pathogen usually associated with periapical infections and it must be bear in mind that the tested solution (herbal extract of *Sapindus mukorossi*) can be modified with some other ingredients for expected appropriate effects.

CONCLUSION

In order to preserve the strength of dentin that will ultimately be beneficial for increase in survival of teeth in function probably would be better option to choose herbal root canal irrigant (*Sapindus mukorossi*) that has displayed characteristic properties for future endeavour and presented as an economic and effective alternative amongst root canal irrigants.

Acknowledgments: The samples for the study were collected from the outpatient department of oral and maxillofacial surgery Dow University of Health Sciences, sample preparation using microtome for tooth sectioning, construction of acrylic blocks and sample analysis by Vickers micro hardness tester were performed in metallurgical department of NED University, herbal extract formulation was done in HEJ research institute University of Karachi.

Conflict of Interest: None to declare

Ethics Approval IRB approval # 2016/221 (has been mentioned in methodology)

Patient Consent: (As it is an in-vitro study, no any patient is involved in-vivo) approved by Ethical review board of Dow University

AUTHORS' CONTRIBUTIONS

Author 1, idea innovation, sample collection, sample preparation, analyses. Data entry, Primary writing

Author 2, Supervised the study, data inferencing

Author 3, Assisted the herbal extract preparation, sample testing procedure

Author 4, Collaborated in sample testing, collaborated in manuscript writing

Author 5, sample size calculation, Data statistical analysis, using statistical software

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Address for Correspondence:

Dr. Asad Farooq, Department of Science of dental materials, Liaquat College of Medicine and Dentistry, Karachi-Pakistan.
Cell: +92 333 320 1843
Email: drasadfarooq@gmail.com