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ORIGINAL ARTICLE

Erosive Potential of 1% Phytic Acid on Radicular Dentine at Different Time Intervals

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ABSTRACT

Objective: The objective of this in-vitro study was to compare the erosive potential and smear layer removal ability of 1% Phytic acid (IP6) and 17% Ethylenediaminetetaacetic acid (EDTA).

Methods: Canal preparation of 225 single rooted extracted human teeth was performed with Protaper NiTi rotary instruments. Teeth were divided into three groups according to the final irrigation protocol. Group 1: Saline irrigation (n=75), Group 2: 17% EDTA (n=75), Group 3: 1% Phytic Acid (n=75). Roots were splitted and observed under Scanning Electron Microscope (SEM) for erosion and smear layer removal. Mean differences between the groups for smear layer removal and erosion were assessed using the Kruskal Wallis and Mann Whitney U test. ($P \leq 0.05$) Friedman and Willcoxon Signed Rank tests were used to make comparisons within the groups.

Results: Group 3 was significantly less erosive than Group 2 at all root portions ($P<0.001$). With regards to smear layer removal, group 2 (EDTA) removed more smear layer compared to group 3 (Phytic acid) at all root portions ($P<0.001$). Both 17% EDTA and 1% IP6 removed significantly less smear layer in the apical root portion. Intra group comparisons revealed no significant differences at any root level. There was a time dependent increase in erosion and smear layer removal in Group 2, with severe erosion at 5 minutes time interval. In Group 3, however, there was moderate erosion and smear removal at 3 and 5 minutes interval.

Conclusion: IP6 at the concentration of 1% and pH 3 was less erosive than 17% EDTA. It exhibited moderate smear layer removal ability.

Keywords: Ethylenediaminetetraacetic acid, erosion, phytic acid, radicular dentine, smear layer

HIGHLIGHTS

- Smear layer removal is required for successful endodontic treatment, however, its complete removal is still questionable.
- Different methods are used for the removal of smear layer among which chelators are widely used.
- EDTA is most commonly used and is a gold standard for removal of smear layer. But it has many short comings; it erodes radicular dentine, decreases its microhardness and is damaging to periapical tissues and to the environment.
- IP6 is a natural chelator and it possess beneficial properties; anticariogenic, antiplaque, reduced toxicity and erosion with considerable smear layer removal ability.
- Owing to its various advantages, 1% Phytic acid is proposed as an alternative chelator.

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INTRODUCTION

Cleaning and shaping of the root canal system is essential for successful endodontic outcome. Mechanical instrumentation results in smear layer formation (1). Removal of smear layer is necessary to achieve successful treatment outcome. Smear layer harbours bacteria (2) and hinders the penetration of different irrigants, intracanal medicaments (3) and sealers used for obturating the root canals (4). There are different strategies for the management of smear layer. Examples include Sonic and Ultrasonic activation, different endodontic file systems, irrigants and chelators (5). Of these, chelators are most commonly used and are relatively effective when used as a final irrigant (6). EDTA is a chelating agent and is considered a gold standard because of its excellent ability to remove smear layer (7). However, EDTA may erode radicular dentine when used as final rinse for more than 1 minute (7), decreases microhardness (8), and has a detrimental effect on periapical tissue (9). In addition, there are concerns about the impact of EDTA on the environment (10).

Phytic acid (IP6) was introduced in 2015 as a natural chelating agent to overcome deleterious effects of EDTA (11). It has shown various benefits in medicine and food industry as a preservative (12). It is claimed to possess anti-cariogenic and anti-plaque effect because of its inherent ability to prevent dissolution of enamel (13, 14). Moreover, it was reported that IP6 is more biocompatible and less cytotoxic than EDTA with considerable smear layer removal ability (15).

On the basis of these advantages, 1% IP6 was proposed as an alternative chelating agent in a recent report (11). Variables such as its effect on microhardness (16), removal ability of intracanal medication (17), effect on dentine bond (18), chelating efficacy (19), calcium loss from root dentine (20), effect on dental pulp stem cells (21), effect on calcium silicate based cements (22), antimicrobial effect (23) and role in regenerative endodontics (24) have been reported previously.

Literature on erosive potential and smear removal ability of 1% IP6 was reported with conflicting results. Kalçay found more erosion of dentine with 1% IP6 as compared to 17% EDTA (25). Nassar et al. reported equal or better smear removal of IP6 as compared to 17% EDTA (11). In contrast, Jagzap et al. reported less effective smear removal by IP6 (26). This contrary evidence warrants a study on IP6 with known pH. Therefore, the objective of this study was to compare the erosive potential and smear layer removal ability of a high pH (pH 3) 1% IP6 with 17% EDTA, using both as final irrigants at different time intervals.

MATERIALS AND METHODS

Sample size calculation

Sample size was calculated with PASS v.11 using two sample t test. Smear layer scores were used from a previously reported study (mean±SD, EDTA 2.3±0.4 and Control 3.5±0.6, CI 99%) (27). The calculated sample was 11 per sub-group which was increased to 25 for statistical reasons. To increase the statistical significance, sample size was raised from 11 to 25.

Study settings and teeth selection

A a total of 225 fully formed permanent extracted human single rooted teeth were collected and stored in 0.1% Thymol at room temperature until use. Collected teeth were observed for presence of any defect and discarded if any of the following were found; caries, fracture, prior endodontic treatment, developmental defects, cracks or root resorption.

The study duration was 12 months and it was conducted at the Department of Operative Dentistry, Dr. Ishrat-ul-Ibad Khan Institute of Oral Health Sciences, Dow University of Health Sciences Karachi. The SEM evaluation was performed at Centralized Laboratory, University of Karachi. The project was approved by an Institutional ethical review board (ref: IRB-809/DUHS/Approval/2016/336).

Randomization and blinding

Teeth were divided into three groups (75 each). Group 1: Saline irrigation, Group 2: 17% EDTA, Group 3: 1% Phytic Acid. Each group was sub-divided into three sub groups (25 teeth each) according to different time intervals. Randomized sequence was generated for treatment. All three irrigating solution 17% EDTA (pH 7) (Meta-MD-Cleanser), 1% IP6 (pH 3) (Sigma-Aldrich) and Saline (control) were blinded and coded.

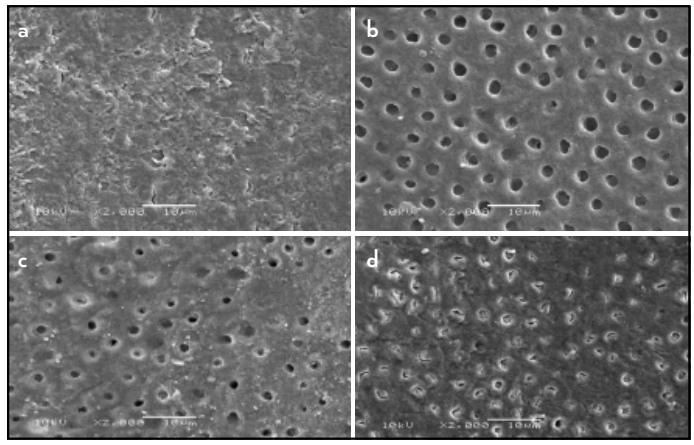

Figure 1. Photomicrographs at X2000 after 1-minute irrigation (a) Group 1 (control group): showing heavy smear layer at all root portions and time intervals (b) Group 2: complete removal of smear layer in coronal root portion (c) Group 2: apical root portion showing less smear layer removal (d) Group 3: showing moderate removal of smear layer from coronal root portion

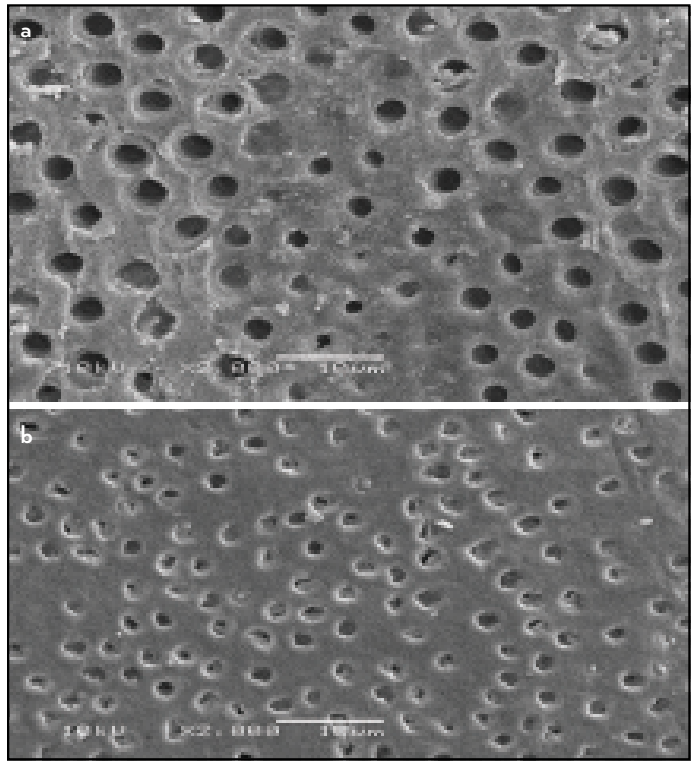

Figure 2. Photomicrographs at X2000 after 3-minutes irrigation (a) Group 2: showing moderate erosion at coronal root portion (b) Group 3: showing moderate removal of smear layer and erosion at coronal root protion

Preparation of 1% IP6

For the preparation of 1% IP6, 1gm of IP6 (Sigma-Aldrich) was added in 100 ml of distilled water and mixture was stirred for two hours using magnetic stirrer (Spectrum MS300HS, Phasi Charoen District, Bangkok). The resultant pH of IP6 was 3 as measured by a pH meter (Adwa AD 1020, Szeged, Hungary).

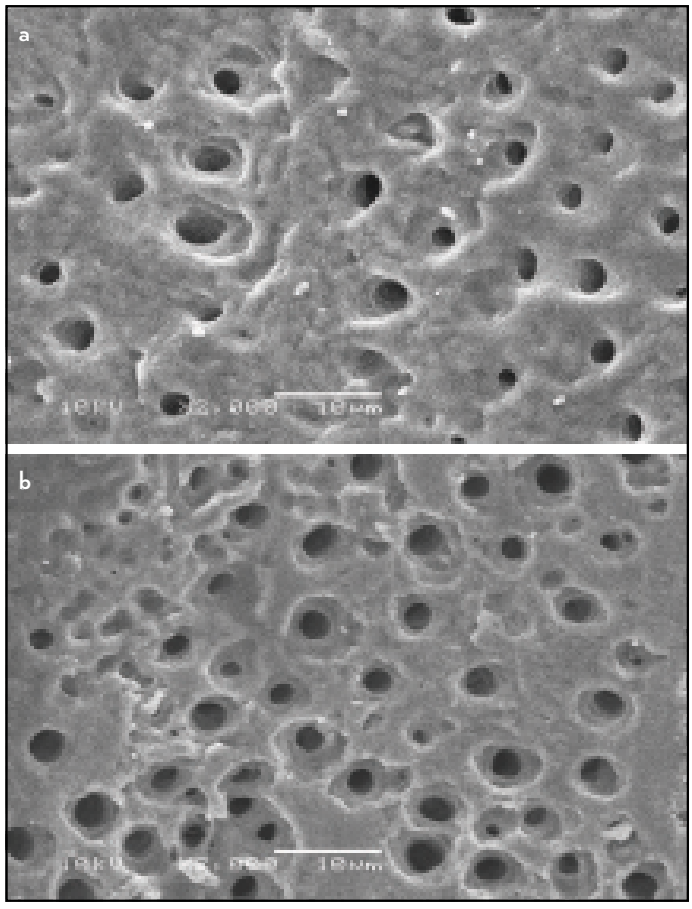

Figure 3. Photomicrographs at X2000 after 5-minutes irrigation. Group 2: severe erosion at (a) coronal and (b) middle root portion

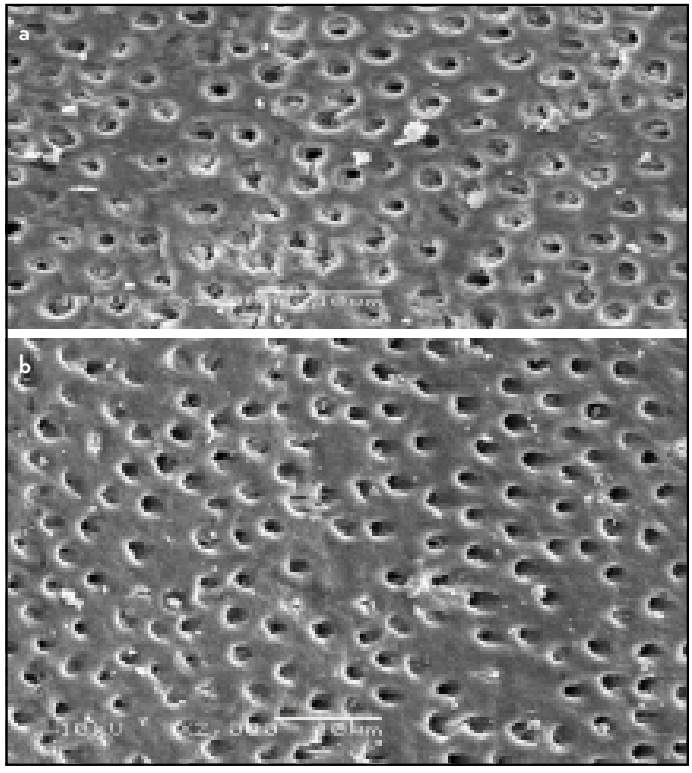

Figure 4. Photomicrographs at X2000 after 5-minutes irrigation. Group 3: moderate smear layer removal at all root portions (a)coronal (b) middle root portion

Endodontic preparation

Crown portion of each tooth was removed at cement-enamel junction using diamond disc. Length was determined by inserting a #10 K file (Mani Inc. Japan) till it was visible at the root apex and 1 mm was subtracted from it. Apex was sealed with two coats of nail varnish to prevent the flow of irrigants and simulate a closed root canal system. Endodontic preparation was performed with Protaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) using the standard sequence till finisher file 3 (F3, tip diameter 0.03 mm, taper 9%). A 30-gauge side vented needle (Diadent) was used for irrigating 3% sodium hypochlorite between each file. All canals were rinsed with 5 ml of distilled water before the application of final irrigation solution.

Final irrigation protocol

Final irrigating solution and time was selected according to the group allocation determined by computer generated sequence as follows:

Group 1: Saline irrigation, 1 minute, 3 minutes and 5 minutes

Group 2: 17% EDTA, 1 minute, 3 minutes and 5 minutes

Group 3: 1% Phytic Acid, 1 minute, 3 minutes and 5 minutes

A 30-gauge side vented needle attached to 5 ml Luer lock syringe was used to deliver final irrigant. Irrigation needle was kept 2 mm short of working length and irrigant was delivered inside the canal while simultaneously moving it in coronoapical direction. All teeth received equal volume (5 ml) of final irrigant.

Sample preparation for SEM evaluation

The canals were irrigated with 5 ml of distilled water and dried with sterile paper point. Two longitudinal grooves were made on buccal and lingual surface of the root with diamond disc. Roots were separated into two halves with chisel and mallet. The half that contained more visible part of apex was used for further analysis.

SEM evaluation

Root specimens were dehydrated and mounted on metallic stubs and coated with gold sputter to make them conductive for SEM (JEOL JSM-6380A, Japan) evaluation. SEM photomicrographs were taken at X 2000 magnification at coronal (10-11 mm to apex), middle (6-7 mm to apex) and apical (1-3 mm to apex) third of each root.

Evaluation of photomicrographs

Blind evaluation was performed independently by two endodontists. Smear layer removal and erosion was scored according to previously published criteria as follows (28).

Smear layer removal:

Score 1: No smear layer (no smear layer on the surface of the root canals with all tubules clean and open).

Score 2: Moderate smear layer (no smear layer on the surface of root canals but tubules contain debris).

Score 3: Heavy smear layer (smear layer covers the root canal surface and the tubules).

Degree of erosion

Score 1: No erosion (all tubules look normal in appearance and size).

Score 2: Moderate erosion (peritubular dentine is eroded).

Score 3: Severe erosion (intertubular dentine is destroyed, and tubules are connected to each other).

Statistical analysis

Spearman's rank-order correlation coefficient was applied to calculate inter-examiner reliability. Statistical comparison for EDTA and Phytic acid was assessed using Mann Whitney test. Friedman and Willcoxon Signed Ranks tests were used to make comparisons within group according to root portions. Mean differences for smear layer removal and erosion were assessed according to different time intervals by using Kruskal Wallis test. P value at<0.05 was considered as significant. Data was analyzed using IBM SPSS version 24 (Armonk, NY: IBM Corp.).

RESULTS

Correlation between both the evaluator's score for smear layer removal was 0.915 (P<0.001) and for erosion was 0.881 (P<0.001) which demonstrated good agreement between the examiners.

Mean smear layer scores

Table 1 represents the description of mean smear layer removal scores of all three groups at coronal, middle and apical portion. Group 2 (17% EDTA) was better than group 3 (1% IP6) at all root portions (P<0.001). Intra group comparison revealed that EDTA and IP6 removed significantly less smear layer in apical root portion than middle and coronal root portions (p-value EDTA<0.001, IP6=0.018).

TABLE 1. Mean smear scores

Irrigant type	Root portions						P-value**
	Coronal		Middle		Apical		
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	
Saline	2.96±0.18	3.00	2.96±0.19	3.00	2.97±0.16	3.00	0.549
EDTA (17%)	1.12±0.36	1.00	1.13±0.37	1.00	1.27±0.54	1.00	<0.001
P-value***		0.564[a]		<0.001[b]		<0.001[c]	
IP6 (1%)	1.56±0.51	2.00	1.56±0.49	2.00	1.68±0.63	2.00	0.018
P-value***		0.869[a]		0.003[b]		0.026[c]	
P-value*	<0.001		<0.001		<0.001		

*P-value calculated using Mann-Whitney analysis, representing significance between EDTA and IP6, **P-value calculated using Friedman test, representing significance in each root portion, ***P-value calculated using Wilcoxon signed-rank test, [a]Coronal vs middle, b middle vs apical, c coronal vs apical

TABLE 2. Mean erosion scores

Irrigant type	Root portions						P-value**
	Coronal		Middle		Apical		
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	
Saline	1.02±0.14	1.00	1.02±0.14	1.00	1.02±0.23	1.00	0.779
EDTA (17%)	2.15±0.83	2.00	2.22±0.80	2.00	2.08±0.85	2.00	0.132
P-value***		0.407[a]		0.059[b]		0.333[c]	
IP6 (1%)	1.49±0.70	1.00	1.53±0.69	1.00	1.58±0.76	1.00	0.443
P-value***		0.426[a]		0.161[b]		0.313[c]	
P-value*		<0.001		<0.001		<0.001	

*P-value calculated using Mann-Whitney analysis, representing significance between EDTA and IP6, **P-value calculated using Friedman test, representing significance in each root portion, ***P-value calculated using Wilcoxon signed-rank test, [a]Coronal vs middle, b middle vs apical, c coronal vs apical

TABLE 3. Smear scores at 1 minute for each irrigant

Irrigant type	Root portions					
	Coronal		Middle		Apical	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Saline	2.96±0.19	3.00	2.92±0.27	3.00	2.92±0.27	3.00
EDTA (17%)	1.20±0.49	1.00	1.24±0.51	1.00	1.48±0.70	1.00
IP6 (1%)	1.70±0.46	2.00	1.64±0.48	2.00	1.80±0.67	2.00
P-value*	<0.001		<0.001		<0.001	

*P-value calculated using Kruskal Wallis analysis, representing significance in each column

TABLE 4. Erosion scores at 1 minute for each irrigant

Irrigant type	Root portions					
	Coronal		Middle		Apical	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Saline	1.04±0.19	1.00	1.04±0.19	1.00	1.08±0.39	1.00
EDTA (17%)	2.02±0.82	2.00	2.10±0.78	2.00	2.04±0.90	2.00
IP6 (1%)	1.24±0.46	1.00	1.38±0.63	1.00	1.50±0.76	1.00
P-value*		<0.001		<0.001		<0.001

*P-value calculated using Kruskal Wallis analysis, representing significance in each column

TABLE 5. Smear scores at 3 minutes for each irrigant

Irrigant type	Root portions					
	Coronal		Middle		Apical	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Saline	2.96±0.19	3.00	2.96±0.19	3.00	3.00±0.00	3.00
EDTA (17%)	1.10±0.30	1.00	1.08±0.27	1.00	1.16±0.37	1.00
IP6 (1%)	1.54±0.54	2.00	1.48±0.50	2.00	1.66±0.65	2.00
P-value*		<0.001		<0.001		<0.001

*P-value calculated using Kruskal Wallis analysis, representing significance in each column

TABLE 6. Erosion score at 3 minutes for each irrigant

Irrigant type	Root portions					
	Coronal		Middle		Apical	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Saline	1.00±0.00	1.00	1.00±0.00	1.00	1.00±0.00	1.00
EDTA (17%)	2.04±0.83	2.00	2.18±0.77	2.00	2.20±0.78	2.00
IP6 (1%)	1.72±0.80	1.50	1.74±0.77	2.00	1.64±0.80	1.00
P-value*	<0.001		<0.001		<0.001	

*P-value calculated using Kruskal Wallis analysis, representing significance in each column

TABLE 7. Smear scores at 5 minutes for each irrigant

Irrigant type	Root portions				
	Coronal		Middle		Apical
	Mean±SD	Median	Mean±SD	Median	Mean±SD
Saline	2.98±0.14	3.00	3.00±0.00	3.00	3.00±0.00
EDTA (17%)	1.06±0.23	1.00	1.08±0.27	1.00	1.18±0.43
IP6 (1%)	1.46±0.50	2.00	1.56±0.50	2.00	1.60±0.57
P-value*	<0.001		<0.001		<0.001

*P-value calculated using Kruskal Wallis analysis, representing significance in each column

Mean erosion scores

Table 2 presents the description of mean erosion scores of all three groups at coronal, middle and apical portion. Group 3 (IP6) was significantly less erosive than EDTA at all root portions (P<0.001). Intra group comparisons revealed no significant differences at any root level. (saline=0.779, EDTA=0.132, IP6=0.443)

Mean smear layer and erosion scores at different time intervals:

At one minute interval

There was an absence of smear layer in group 2 from coronal and middle but tubules contained debris in apical root potion. Group 3 was moderately effective in all root portions showing no smear layer on surface but some tubules were filled with

TABLE 8. Erosion score at 5 minutes for each irrigant

Irrigant type	Root portions					
	Coronal		Middle		Apical	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Saline	1.02±0.14	1.00	1.02±0.14	1.00	1.00±0.00	1.00
EDTA (17%)	2.40±0.80	3.00	2.38±0.83	3.00	2.02±0.86	2.00
IP6 (1%)	1.52±0.67	1.00	1.48±0.61	1.00	1.62±0.72	1.00
P-value*	<0.001		<0.001		<0.001	

*P-value calculated using Kruskal Wallis analysis, representing significance in each column

debris (P<0.001) (Table 3). There was moderate erosion in all root portions of group 2, while in group 3 there was no erosion. (P<0.001) (Table 4).

At three minutes interval

After 3 minutes, there was complete smear layer removal from all root portions in group 2. Group 3 had moderate amount of smear but more tubules were open containing less debris than at 1 minute (Table 5). Moderate erosion was observed in both group 2 and 3 at all levels (P<0.001) (Table 6).

At five minutes interval

There was complete smear removal in group 2 (Table 7), however there was severe erosion at coronal and mid-root level (P<0.001) (Table 8). While in group 3, there was moderate erosion at all root levels.

DISCUSSION

This study compared dentine erosion and smear removal using a high pH IP6 (1%) and 17% EDTA as a final irrigant at 3 and 5 minutes interval. Time and pH can influence erosive and chelating ability of an irrigating solution (29). Results of this study demonstrated that 1% IP6 was moderately effective in removing smear layer and was less erosive than 17% EDTA. The results differ from the findings of Nassar et al. (11) but are in accordance with Jagzap et al. (26) in terms of smear layer removal. In addition, when erosion was evaluated, we found conflicting results with the findings of Kalcay and Tinaz (25).

The two studies on smear removal ability of IP6 reported the potential of IP6 as an alternate to EDTA for smear layer management (11, 15). The former study (11) suggested that IP6 was more effective than EDTA when used on flat dentine disks but of equal effectiveness when used in closed root canal system. Both of these results were contradictory to our findings which could be due to flat dentine disks used by Nassar et al. (11). Whereas decreased penetration and low turnover of irrigant in a closed root canal system used in our study can be attributed to differences in results. In addition, pH of IP6 used by Nassar et al. (11) was 1.3 while the current study used a more basic solution with pH 3. Therefore, a higher pH may be responsible for more smear removal. The effect of pH on erosive and chelating ability of an irrigating solution have been reported previously (29).

There was incomplete smear layer removal at the apical portion in group 2 and 3 which is in accordance with two studies which found that both 17% EDTA and 1% IP6 were unable to completely clean the apical portion of the root (11, 26). A possible explanation for this could be a decrease in dentinal tubular density from coronal to apical direction and presence of more sclerosed dentinal tubules. Also use of needle syringe irrigation in closed root canal system may be incapable of cleaning the apical root part. Another study also demonstrated that canal cleaning ability of ultrasonic activation was questionable in apical third due to complex anatomy (30). However, negative pressure irrigation may improve irrigation effectiveness in root canal systems (31). When results were compared at 1, 3 and 5 minutes, 17% EDTA adequately removed smear layer, however IP6 was found to have a moderate effect on smear. There was a time dependent improvement in its smear removal ability.

One study reported more erosion with 0.5 and 1% IP6 when used for 1 minute which is conflicting with our findings (25). The difference in the results can be attributed to the irrigation regime followed by that study which used sodium hypochlorite as final rinse after thoroughly rinsing the canal with the chelator. The use of sodium hypochlorite after EDTA as a final rinse was reported to cause erosion of dentine irrespective of the type of chelator used (7). In addition, that study (25) used an open system as opposed to a closed root canal system in our study which may partly explain the difference in findings. An open system may allow more fresh irrigant to reach the apical open third and hence may allow greater turnover of irrigant causing more erosion due to availability of fresh chelator (31). However, our closed system better mimics natural condition since periapical tissue pressure does not allow the irrigant to pass out of canal system easily.

Nikhil et al. reported that 1% IP6 has less impact in reducing microhardness as compared to 17% EDTA(16). Hence, we recommend the use of 1% IP6 root canal chelator which is extracted from natural sources and has reduced detrimental effect on root dentine.

Further studies should be conducted to better understand the effect of pH of IP6 on smear removal and dentine erosion. Also the use of 1% IP6 in curved canals and the measurement of micro-hardness and fracture resistance with other contemporary irrigating solutions should be evaluated.

CONCLUSION

IP6 at the concentration of 1 % was less erosive than 17% EDTA and exhibited moderate smear layer removal ability.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by an Institutional ethical review board (ref: IRB-809/DUHS/Approval/2016/336).

Peer-review: Externally peer-reviewed.

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