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Evaluation of efficacy of three herbal extracts on smear layer removal during root canal preparation using scanning electron microscope: An in vitro study.

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Abstract

Aim: To evaluate the efficacy of three herbal extracts Salvadora persica (S.persica), Triphala, Citrus aurantifolia (CA) on smear layer removal using scanning electron microscope.

Materials and Methods: Fifty extracted human premolar teeth, indicated for extraction due to orthodontic/periodontal reasons were selected in the study. The teeth were decoronated to obtain a standardized root length of 15 mm. Root canals were prepared with the crown-down technique by using ProTaper NiTi rotary instruments The teeth were then randomly divided into three experimental (n=30), one positive control group (n=10) and one negative control group (n=10). Group A: Saline, Group B: 17% EDTA solution, Group C: Salvodera persica, Group D: Triphala, Group E: Citrus

aurantifolia. The canals were irrigated for 5min with 5ml of respective groups. The roots were then split into two halves with a chisel and evaluated the smear layer using scanning electron microscope. The score data for the presence or absence of the smear layer were statistically analyzed by Kruskall–Wallis ANOVA analysis with post-hoc Conover test. All statistical analyses were set with a significance level of $p< 0.05$.

Results: There was a significant difference in the mean smear values among the five groups at coronal site, middle site and apical site. At coronal site, post-hoc test showed that group 1 had significantly higher mean smear layer score than group 2, 3, 4 and 5. At middle site, post-hoc test showed that group 1 had significantly higher mean smear layer score than group 2, 3, 4 and 5. At apical site, post-hoc test showed that group 1 had significantly higher mean smear layer score than group 2, 3, 4 and 5.

Conclusion: None of the irrigation solutions tested was capable of fully removing the smear layer from the apical thirds. 17% EDTA was better in all the sites (coronal, middle, apical) when compared with S.persica (5mg/ml), Triphala (5mg/ml) and Citrus aurantifolia (5mg/ml) extracts.

Keywords: Citrus aurantifolia, Salvadora Persica, Scanning electron microscope, Smear layer, Triphala

Introduction

Diagnosis, instrumentation, obturation and restoration are the main steps involved in the treatment of teeth with pulpal and periapical diseases. Elimination or significant reduction of irritants and prevention of recontamination of the root canal after treatment are the essential elements for successful outcomes.[1]

Root canal cleaning means removing all potential irritants such as bacteria and their byproducts, organic/inorganic debris, vital and necrotic pulp tissues, as well as blood. Acceptable cleaning of the root canal can be achieved through irrigation and instrumentation. [2] One of the main purposes of cleaning and shaping the canal system is to maintain long term success after the root canal therapy.[3]

During the process of instrumentation, large amount of dentin debris mix with vital and necrotic remnants of pulp tissue, and in combination with microorganisms and microbial toxins adhered to the root canal wall, form a smear layer.[4] However there is some controversy regarding the removal of the smear layer in dentinal tubules.[5] Latest evidence showed that the smear layer inhibits the penetration of antimicrobial irrigants and medication into the dentinal tubules.[6,7] Therefore, for closer adherence of obturants to the root canal wall and to reduce the apical as well as coronal micro-leakage, the smear layer should ideally be removed.[8,9]

Various irrigants are used for root canal treatment.[5] Sodium hypochlorite (NaOCl), one of the most popular irrigants in endodontics, has strong antimicrobial activity and organolytic effects, however it cannot remove the smear layer .[10,11,12] The smear layer mainly consists of inorganic substances which are soluble in acids. Various types of chelating agents like Ethylenediaminetetraacetic acid (EDTA), Citric acid, tannic acid and poly acrylic acid are suitable chemicals for the smear layer removal.[13] Goldman et al. confirmed that a final flush with 17% EDTA followed by NaOCl will completely remove the smear layer.[14,15]

EDTA is mostly used for the smear layer removal; some studies have shown that it cannot effectively remove the smear layer in the apical third of the root canal.[11,16] However, irrigation with EDTA followed by NaOCl could demineralize the dentine and produce erosions in coronal as well as the middle part of the root canal.[14,17]

In dentistry Phytomedicines has been used as anti-inflammatory, antibiotic, analgesic and sedative agents. In endodontics because of the cytotoxic reactions of the most

of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules, trend of recent medicine attends to use biologic medication extracted from natural plants. The advantages of using herbal extracts in endodontics are that they have few side effects, less expensive, better tolerated by patients and renewable in nature.[18] Herbal extracts such as Salvadora persica (S.persica), Triphala, Citrus aurantifolia (CA) have active components like alkaloids, volatile essential oils, glycosides, resins, tannins, citric acid, etc which exert an antimicrobial & chelating properties.[19,]

Very few studies are present in the literature on efficacy of herbal extracts in removal of the smear layer. Hence the present study was undertaken to analyze the efficacy of S.persica, Triphala and CA on the smear layer removal.

Materials and Methods

Fifty extracted human premolar teeth, indicated for extraction due to orthodontic/periodontal reasons were selected in the study. The teeth were cleaned using ultrasonic scaler (Woodpecker Dte D1) followed by sterilization under autoclave (Unique Clave C-79), (Confident Dental Equipment's Ltd).

Inclusion criteria

(1) The apical foramen was fully developed and had no destruction

(2) None of the teeth had received restorative or endodontic therapy

(3) The degree of root canal curvature was confirmed to be within 20 degree measured by the Schneider's method.

Exclusion criteria

(1) Root with more than one canal;

(2) Root with calcified canal;

(3) The degree of root canal curvature more than 20 degree measured by the Schneider's method

(4) Root with open apex.

The teeth were radiographed to confirm root canal patency and the absence of a complicated root canal anatomy. Thereafter, teeth were stored in distilled water until use. The teeth were decoronated to obtain a standardized root length of 15 mm. ISO #10 K-files *(*Dentsply Maillefer*)* were inserted into the root canal until they were just visible at the apical foramina. The working lengths were measured by deducting 1 mm from the length recorded from this point. Root canals were prepared with the crown-down technique by using ProTaper NiTi rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's manual. All samples were prepared to F3 size. Canals were enlarged apically with a ProTaper size 30, 0.09 taper instrument. The root canals were flushed with 1 ml of a 3% NaOCl solution (Nimai Dento India) between the instrument changes using a disposable syringe with a 30-gauge needle (Biodent company, side vent irrigation needle) inserted at a distance of 2 mm from the working length without binding. The teeth were then randomly divided into three experimental (n=30), one positive control group (n=10) and one negative control group (n=10). All the groups were color coded at the root apex with different colored nail polish.

Group A: Saline (Tam-Bran Pharmaceuticals Limited).

Group B: 17% EDTA solution (Ramen Research Product).

Group C: Salvodera persica (S.persica) (5mg/ml concentration).

Group D: Triphala (5mg/ml concentration).

Group E: Citrus aurantifolia (5mg/ml concentration).

The canals were irrigated for 5min with 5ml of respective group. The irrigation solutions were delivered in a passive manner using in and out movements via a sterile 30-guage needle that penetrated within 2mm of the working length. The root canals then underwent a final flush with 5ml of distilled water and dried with paper points.

Two longitudinal grooves were prepared on the buccal and lingual surfaces of each root using a diamond disc (Deccan Dental Plus) without penetration into the canal. The roots were then split into two halves with a chisel (GDC). For each root, the half containing the most visible part of the apex was conserved and coded. The roots were grooved to three levels at 4, 8, and 12 mm from the root apices using a diamond bur (Mani) to define the coronal, middle, and apical thirds. The specimens were left to dry overnight. The coded specimens were then mounted on metallic stubs, gold sputtered, examined and photographed by a scanning electron microscope.

In the present study the scanning electron microscope evaluation was done in Department of Physics, Osmania University, Hyderabad, using SEM EVO MA 15. All specimens were observed under 2000x magnification, with EHT – 20.00kV. The photographs were saved and analyzed for the absence or presence of smear layer. The cleanliness of each root was evaluated at three areas (coronal, middle and apical) by means of a numerical evaluation scale scoring system consisting of following five criteria according to Hulsmaan M et al.

Numerical evaluation (scoring system):

1. SCORE 1 – No smear layer, dentinal tubules open.
2. SCORE 2 – Small amount of smear layer, some dentinal tubules open.
3. SCORE 3 – Homogenous smear layer covering the root canal wall, only few dentinal tubules open.
4. SCORE 4 – Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.
5. SCORE 5 – Heavy, in homogeneous smear layer covering the complete root canal wall.

The scoring procedure was implemented by two examiners who performed the blinded evaluations independently. The reliability of the intra-examiner and inter-examiner results was verified by using the kappa test. The score data for the presence or absence of the smear layer were statistically analyzed by Kruskall–Wallis ANOVA analysis with post-hoc Conover test. All statistical analyses were set with a significance level of $p < 0.05$.

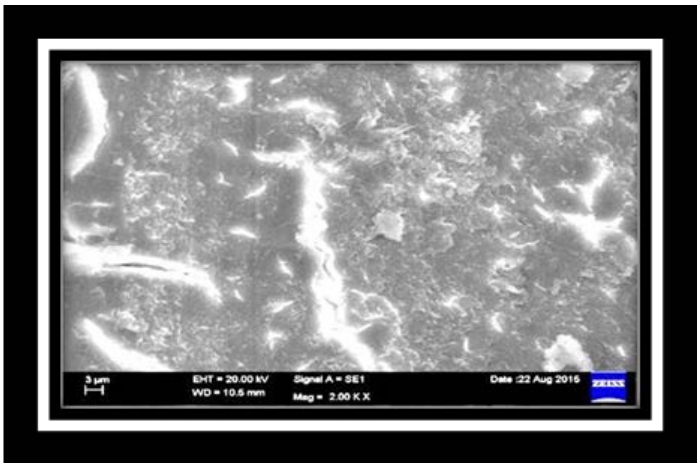

Figure 1a: Saline

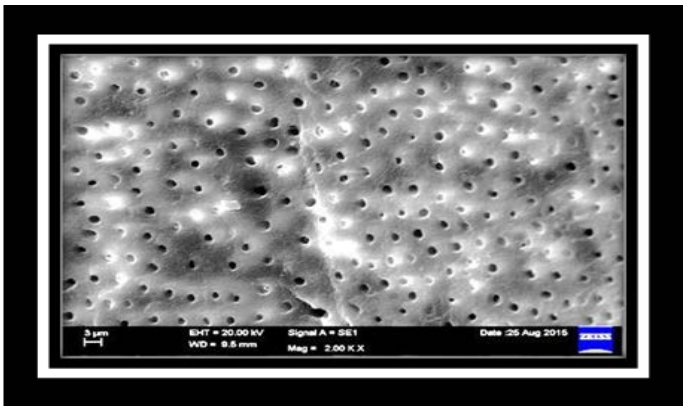

Figure 1b: 17% EDTA

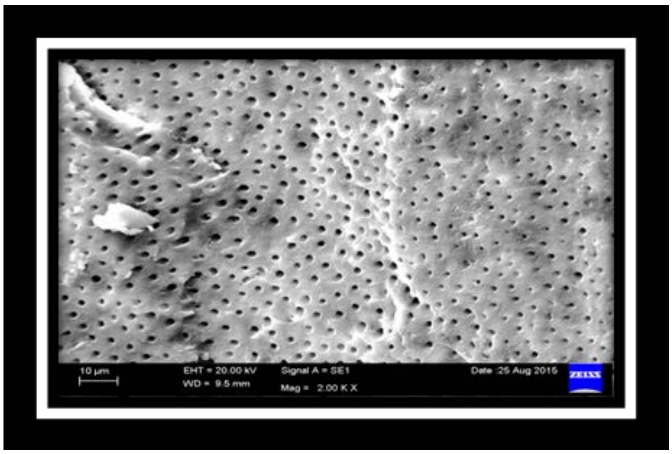

Figure 1c: Salvodera Persica

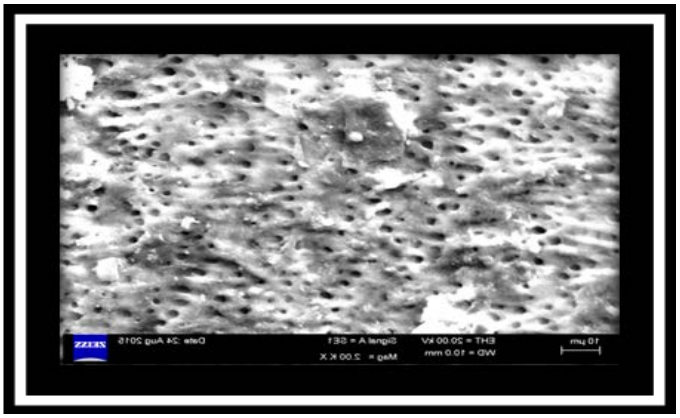

Figure 1d: Triphala

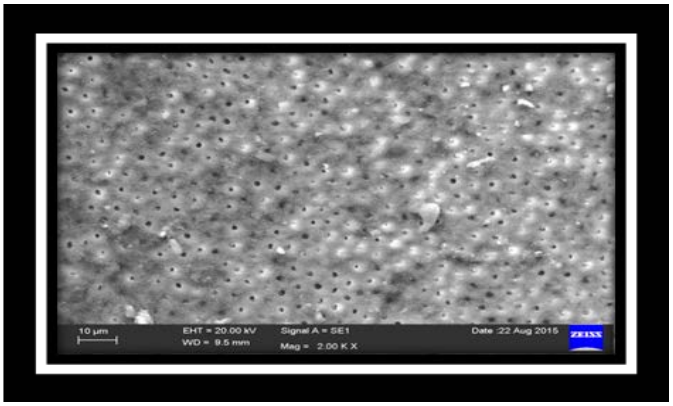

Figure 1e – Citrus aurantifolia

Figure 1: SEM photomicrographs of the coronal third of root canal walls after final irrigation. (@ 2,000 X).

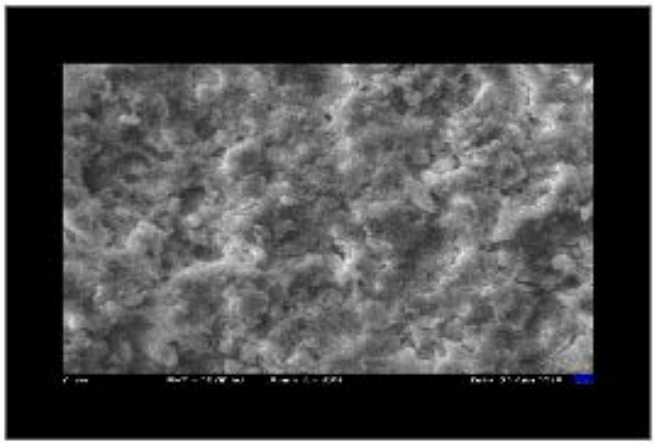

Figure 2a: Saline

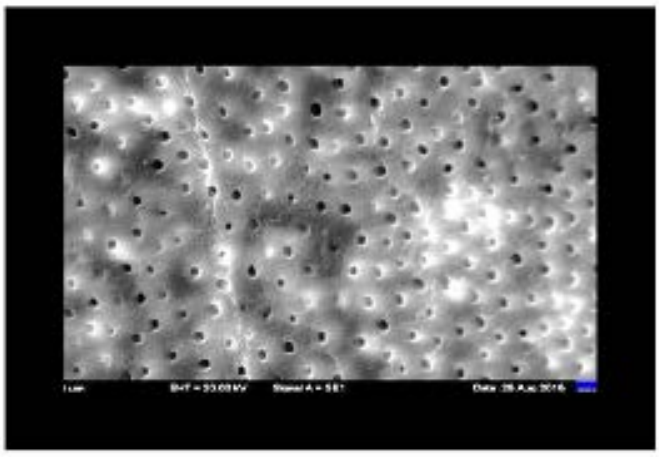

Figure 2b: 17% EDTA

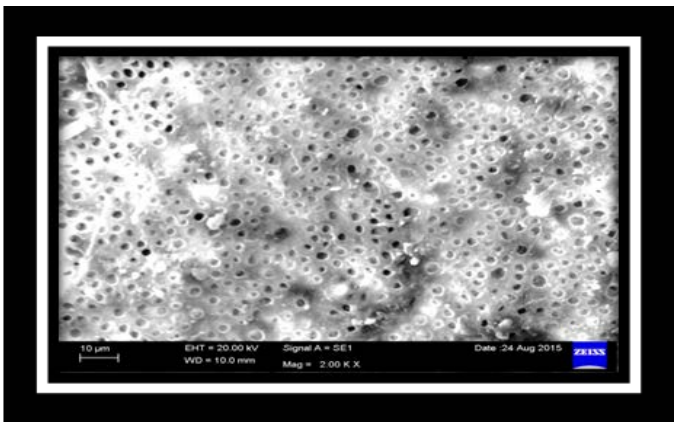

Figure 2c - Salvodera Persica

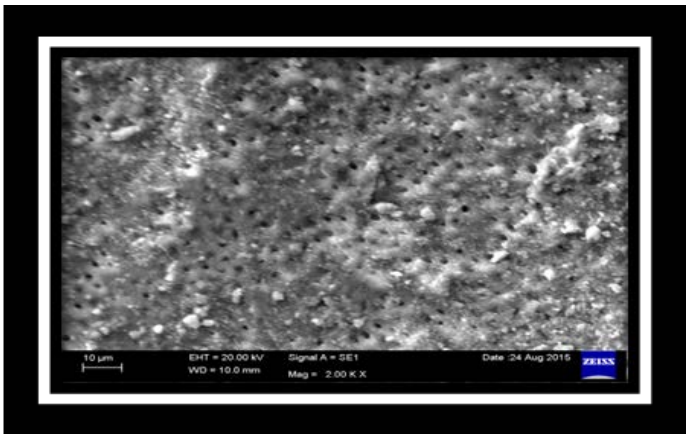

Figure 2d: Triphala.

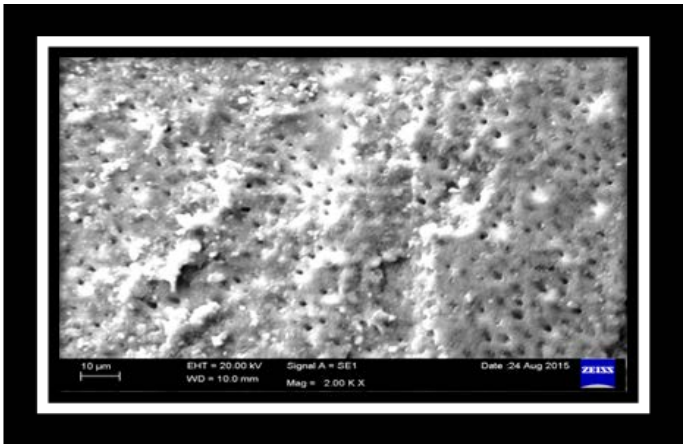

Figure 2e: Citrus aurantifolia

Figure 2: SEM photomicrographs of the middle third of root canal walls after final irrigation. (@ 2,000 X).

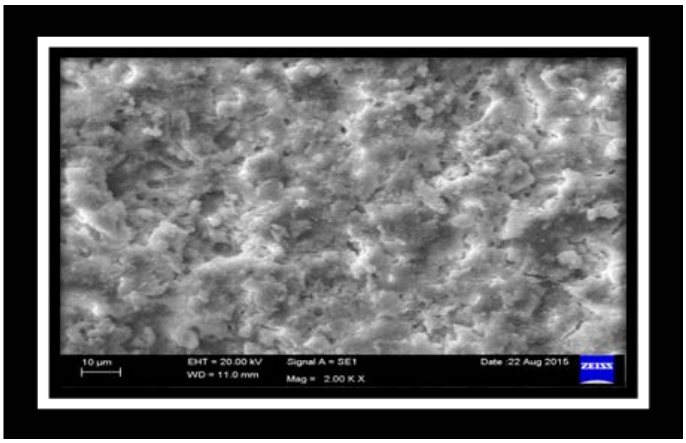

Figure 3a: Saline

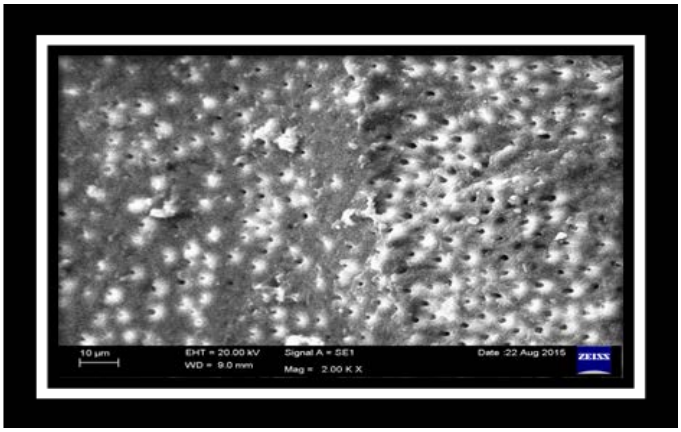

Figure 3b: 17% EDTA

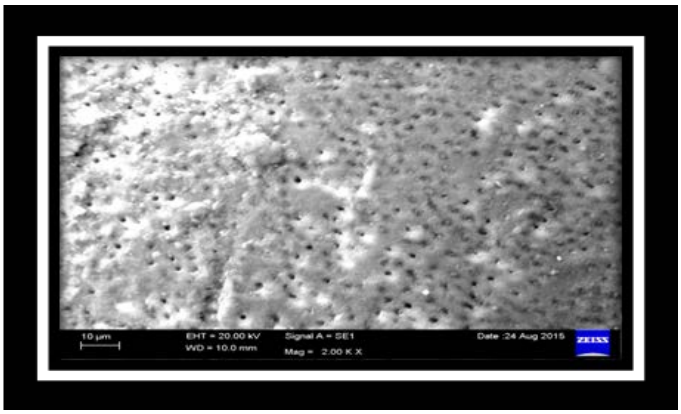

Figure 3c: Salvodera Persica

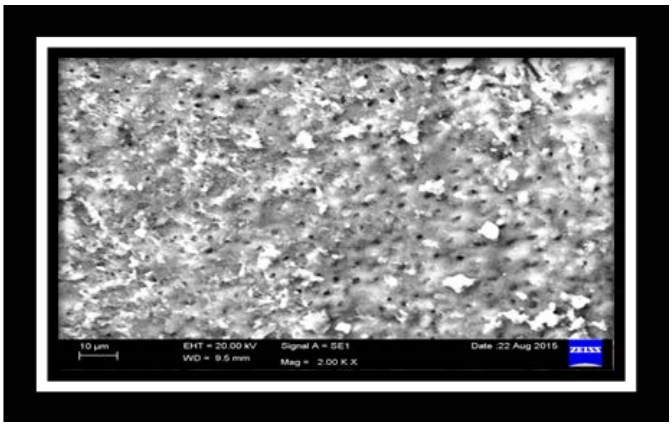

Figure 3d :Triphala

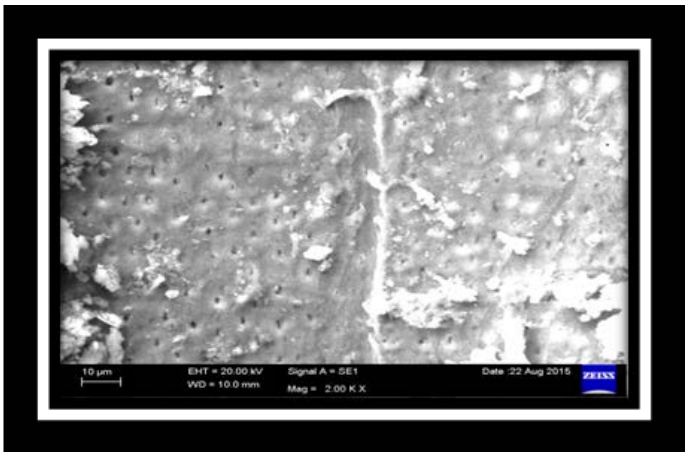

Figure 3e: Citrus aurantifolia

Figure 3: SEM photomicrographs of the apical third of root canal walls after final irrigation. (@ 2,000 X).

Results

There was a significant difference in the mean smear values among the five groups at coronal site, middle site and apical site. At coronal site, post-hoc test showed that group 1 had significantly higher mean smear layer score

than group 2, 3, 4 and 5. Similarly, group 4 and 5 had significantly higher mean smear layer score than group 2 and 3.(Table 1) At middle site, post-hoc test showed that group 1 had significantly higher mean smear layer score than group 2, 3, 4 and 5. Similarly, group 4 and 5 had significantly higher mean smear layer score than group 2 and 3.(Table 2) At apical site, post-hoc test showed that group 1 had significantly higher mean smear layer score than group 2, 3, 4 and 5. Similarly, group 3, 4 and 5 had significantly higher mean smear layer score than group 2.(Table 3)

Table 1: Comparison of efficacy in smear layer removal from coronal third of root canal walls by different groups

Site	Group										p-value	Post-hoc test
	Saline [1]		EDTA [2]		S. Persica [3]		Triphala [4]		CA [5]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Coronal	4.55	.50	1.30	.47	1.45	.66	2.80	.67	2.40	.48	<0.001; Sig	1>2,3,4,5 4,5>2,3,

Table 2: Comparison of efficacy in smear layer removal from middle third of root canal walls by different groups

Site	Group										p-value	Post-hoc test
	Saline [1]		EDTA [2]		S. Persica [3]		Triphala [4]		CA [5]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Middle	4.75	.35	1.65	.42	2.10	.59	3.40	.52	3.15	.78	<0.001; Sig	1>2,3,4,5 4,5>2 4,5>3

Table 3: Comparison of efficacy in smear layer removal from apical third of root canal walls by different groups

Site	Group										p-value	Post-hoc test
	Saline [1]		EDTA [2]		S. Persica [3]		Triphala [4]		CA [5]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Apical	4.75	.35	2.15	.44	3.10	.33	3.80	.53	3.70	.39	<0.001; Sig	1>2,3,4,5 3,4,5>2

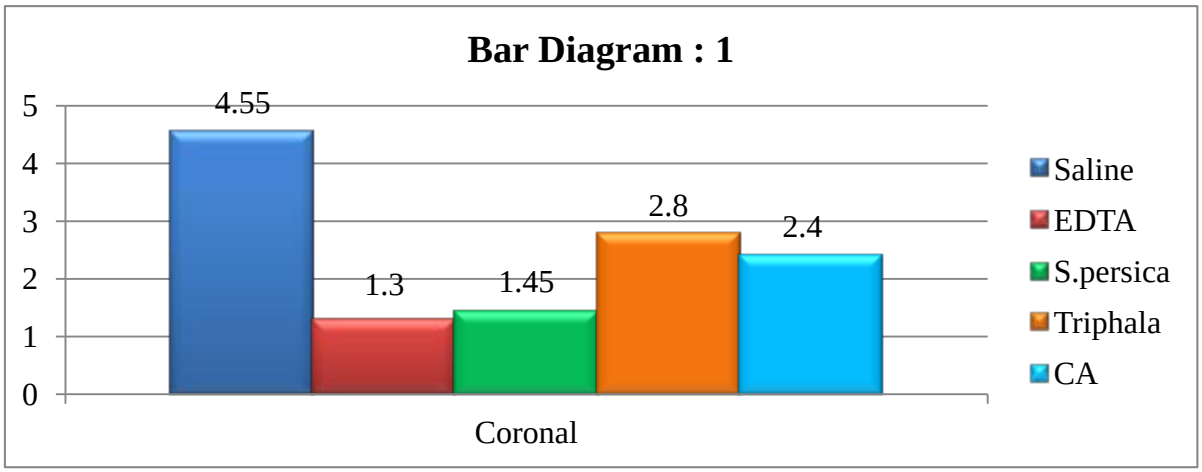

Figure 1 : Mean scores of smear layer present in coronal thirds of the root canal wall

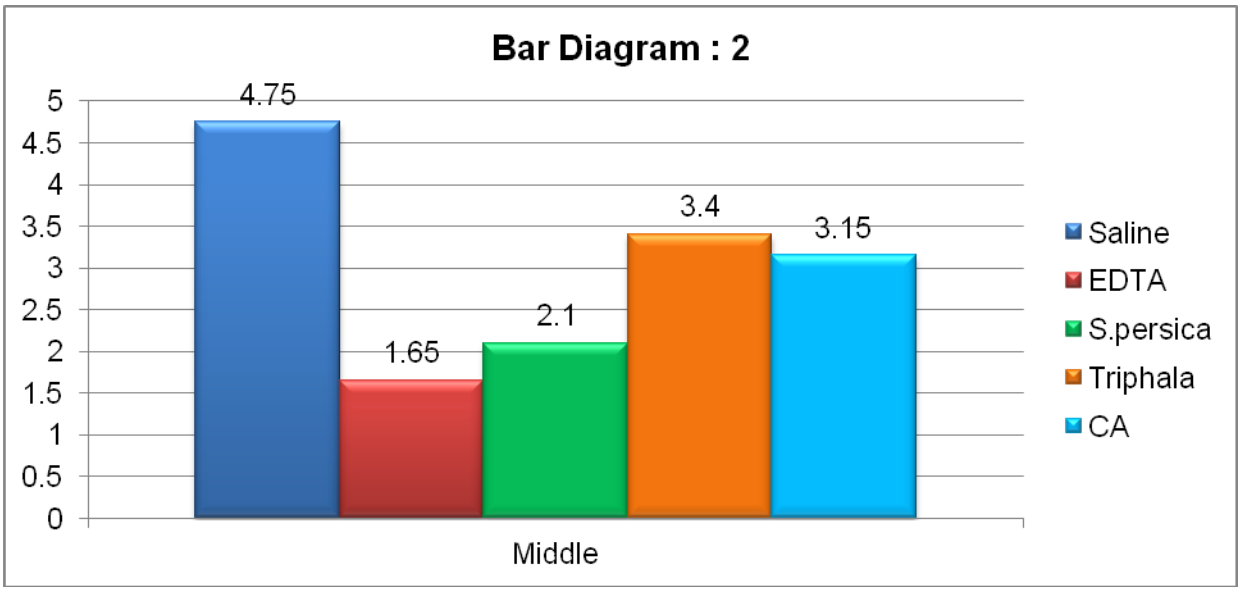

Figure 2: Mean scores of smear layer present in middle thirds of the root canal wall

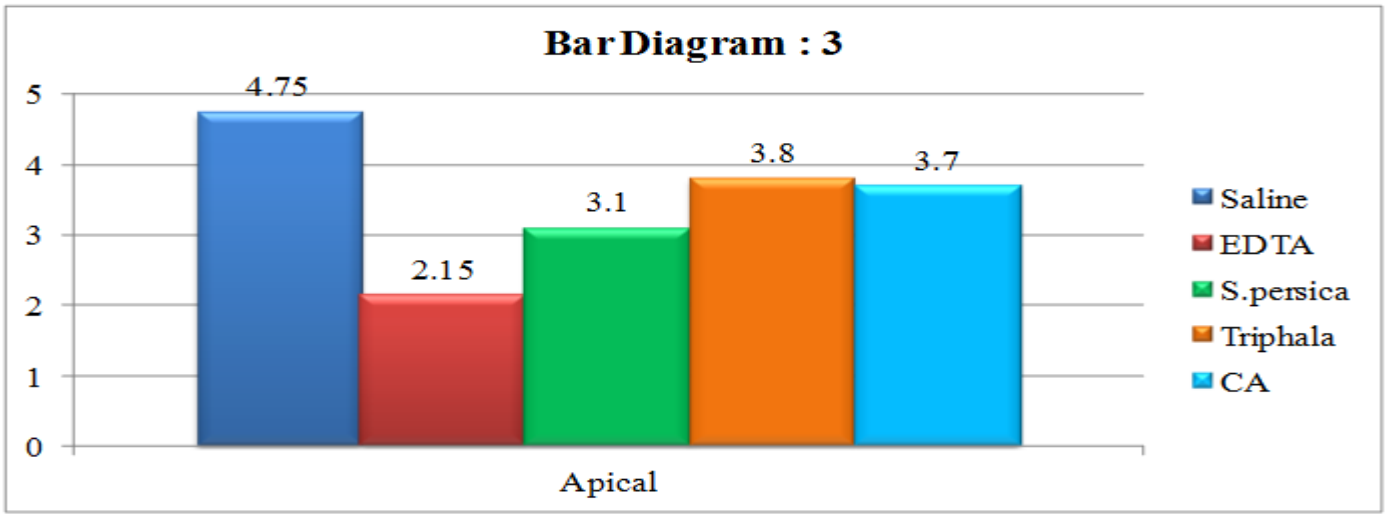

Figure 3: Mean scores of smear layer present in apical thirds of the root canal wall

Discussion

The major goal of root canal treatment is removal of microorganisms from the complex root canal system; it would therefore appear that 'shaping to facilitate cleaning and filling' might be a more appropriate concept.[22]

Whenever dentine is cut using hand or rotary instruments, the mineralized tissues are not shredded or cleaved but shattered to produce considerable quantities of debris. Much of this, made up of very small particles of mineralized collagen matrix, is spread over the surface to form what is called the smear layer.[23]

Although there is controversy concerning whether to remove or retain the smear layer, some reports have suggested that there is no significant difference in leakage with or without the root smear layer (Violich and Chandler, 2010). On the other hand, a recent systematic review and meta-analysis of in vitro leakage studies by Shahravan et al. (2007) concluded that smear layer removal improves the fluid-tight seal of the obturated root canal system.[19]

Regardless of the instrumentation technique or system used, the use of irrigants is essential for debridement of the canal system. Consequently the preparation of root canal systems involves both mechanical and chemical components; hence the concept of 'chemo-mechanical' preparation.[22]

A plethora of irrigants have been used for root canal irrigation. Currently, the most widely used irrigant is NaOCl, which has both antibacterial and tissue-dissolving properties.[22,44] However, sodium hypochlorite is not effective to remove smear layer. It has very little effect on this layer, removing only organic matter. In order to remove inorganic components of the smear layer it is necessary the use of auxiliary irrigating solutions.[24] Smear layer removals requires a combination of NaOCl (an organic solvent) and acids such as, citric acid, tannic, polyacrilic, or phosphoric acid, or chelating agents such as EDTA for the removal of the inorganic part.[25]

Unfortunately, no irrigation solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer. Presently, sodium hypochlorite can be combined with EDTA to offer bactericidal, solvent, and chelating actions (Baumgartner and Mader, 1987).[19] The problem of resistance of microorganisms to antimicrobial drugs is one of the world's current challenges. On the other hand, plant-based antimicrobials are attractive as they are often devoid of many side effects associated with synthetic antimicrobials. The search for new antimicrobial compounds from natural sources is, thus, an ongoing one (Parekh et al. 2005).[26] Various natural plant extracts have antimicrobial properties & chealating effects suggesting their potential to be used as an endodontic irrigant. The advantages of using herbal extracts in endodontics are that they have few side effects, less expensive, better tolerated by patients and renewable in nature.[27]

Herbal extracts such as S.persica, Triphala, and Citrus have active components like organic acids, alkaloids, volatile essential oils, glycosides, resins, tannins, etc which exert an antimicrobial & chelating properties. Hence, the present study was undertaken to analyze the efficacy of S.persica, Triphala and CA on smear layer removal.[19,20,21]

In the present study we chose S.persica because the clinical interest of S.persica arises from a number of mechanisms, including its acidic and antimicrobial properties. By the isolation of the active ingredient from S. persica, Wolinsky and Sote (1983) found that limonoid had a great antimicrobial activity against various Gram positive and Gram negative microorganisms. In this study, an ethanolic S. persica extract solution was used because it has been reported to have a significant antimicrobial effect

against aerobic and anaerobic bacteria when used as a root canal irrigant (Al-Sabawi et al., 2007).[19]

We chose ethanolic extracts of Triphala because it exhibited a broad-spectrum antimicrobial activity against all the microorganisms.[28] Triphala is very good chelating agent, contains fruits that are rich in citric acid that may aid in removal of the smear layer (Prabhakar et al 2010.Kamat S et al 2011, Bhargava K et al 2015).[29,30] We chose CA (lime juice) extract as the irrigant, because it has citric acid, it is able to remove the smear layer and open the dentinal tubules.[31,32]

It has been demonstrated that rotary crown down technique is very effective, creates predictable shaping with significantly less time than conventional technique.[33] According to Khademi et al. (2006) minimum instrumentation size needed for penetration of irrigants to the apical third of the root canal is a #30 file. Therefore, 0.30 mm apical size was chosen for this study.[34]

The 30-gauge needles with side opening and apical opening presented better ability to remove contrast medium from the apical third after endodontic irrigation at all stages of root canal widening. Thus in the present study 30-G needles were used as an endodontic irrigation needles.[35]

The accepted application time of the chelating solution, as supported by the literature, is between 1 and 5 min (Yamada et al., 1983; Torabinejad et al., 2003b). Thus, irrigation for 5 min with irrigant followed by a final rinse with deionized water was adopted for this study.[19]

David Pashley suggested that the smear layer is a very thin layer and is soluble in acids and hence the smear layer will not be apparent on routinely processed specimens examined with a light microscope. The scanning electron microscope has proved a valuable method for assessment of the ability of endodontic procedures to remove debris from pulp space.[33,45]

In the present study, the results showed that 17%EDTA is able to remove the smear layer completely only in the coronal and middle parts of root canal. This concurs with Takeda et al and Parbhu et al.[17,36] Their results also showed that 17%EDTA was not able to produce the expected smear-free surfaces in the apical part of the canal because the apical part was less accessible than the coronal and middle parts for deeper penetration of EDTA. This can probably be explained to the fact that dentin in the apical third is much more sclerosed and the number of dentinal tubules present there is less. It may also not have such a pronounced action at the narrow apical portion as in the middle third.[10]

In the present study the S. persica extract was as effective as 17% EDTA in removing the canal-wall smear layers from the coronal third.[37,38] At the apical third, S. persica extract was statistically less effective than EDTA in removing the smear layer. These results were consistent with the general finding from the endodontic literature that the apical third of the canal is more difficult to clean (Goldman et al., 1982; Barkhordar et al., 1997; O'Connell et al., 2000; Calt and Serper, 2000).[14,39,40,41] However, these results could be due to tubular sclerosis, which is most pronounced in the apical third of the root canal (Vasiliadis et al., 1983).[42]

In the present study there was significant difference between 17%EDTA and CA juice extract in removing the smear layer from coronal, middle and apical parts of root canal. Takeda et al, Mancini et al have proved that citric acid with 6% and 42% concentration, respectively, were not able to remove the smear layer in the apical and middle parts.[16,32,43] In the present study the results disagree with other studies that have indicated that citric acid (with 7% and 10%concentration) removes the smear layer in all parts of the root canal. However, the concentration of citric acid used in their study was higher.

On the other hand, the solution showing the least efficacy in smear layer removal was Triphala, which could not remove the dentinal surface smear layer accumulation completely in all the teeth evaluated. This may be because Triphala of consists of Terminalia bellerica and Terminalia chebula along with Emblica officinalis. This combination in Triphala may increase its pH to alkaline, whereas the pH of Emblica officinalis is 2.8-4.5. Bhargava et al. compared the efficacy of Emblica officinalis and Triphala, in which Emblica officinalis showed the best smear layer removing ability than Triphala. The superior efficacy of smear layer removal with Emblica officinalis could be a result of its low pH.[30]

Scanning electron microscopic pictures of saline group revealed the presence of extensive sludge layer made up of residual organic debris and smear layer. In electron microscopic view (×2000), the presence of heavy smear layer was clearly noticed at all three root thirds, invariably in all samples. It is better to conclude among the three herbal groups that the superior efficacy of smear layer removal with S.persica could be a result of its stearic acid compound and low pH. [19] As the pH increases, the availability of calcium ions from hydroxyapatite for chelation decreases. At the same time, a greater dissociation of the acidic irrigant produces an increased attraction for calcium ions.[30]

Further trends of studies need to investigate the effect of these alternative irrigants on radicular dentin. It is possible that these irrigants could exhibit substantivity with the root dentin. This could be extremely beneficial in maintaining the bacteriostatic environment of the root canal. However, the interaction between these irrigants and root canal sealers also needs to be investigated in subsequent works. Further clinical trials and investigations are required to be considered as effective alternatives to the synthetic root canal irrigants.

Conclusion

None of the irrigation solutions tested was capable of fully removing the smear layer from the apical thirds. 17% EDTA was better in all the sites (coronal, middle, apical) when compared with S.persica (5mg/ml), Triphala (5mg/ml) and Citrus aurantifolia (5mg/ml) extracts. At coronal thirds, the smear layer removing abilities of S.persica (5mg/ml) were found to be as good as 17%EDTA, which was followed by CA (5mg/ml) and Triphala (5mg/ml). At Middle thirds, the smear layer removing abilities of S.persica (5mg/ml) were found to be as good as 17%EDTA, which was followed by CA (5mg/ml) and Triphala (5mg/ml). At apical thirds, S.persica (5mg/ml) was less effective than 17%EDTA in removing the smear layer. S.persica (5mg/ml) was better than CA (5mg/ml) and Triphala (5mg/ml) in removal of the smear layer. CA (5mg/ml) was better than Triphala (5mg/ml) in removal of the smear layer. 17%EDTA was found to remove smear layer significantly more than S.persica (5mg/ml), CA (5mg/ml) and Triphala (5mg/ml).

Limitations of the Present Study

1. As the present study was an in-vitro study done on extracted teeth, the organic and inorganic composition of the tooth may vary from that of natural tooth present in the oral cavity.
2. Working in-vivo is more complex because the variance of root canal anatomy is higher than in a controlled in-vitro experiment.
3. There is scarce information on the quality, safety and greater efficiency of these products for use in endodontics. As most of the studies are carried out ex-vivo, more of these compounds should be subjected to animal and human studies to determine their effectiveness, side effects, toxicity and drug interactions.

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