Research Article

Comparative Evaluation of Different Irrigating Solutions on Smear Layer Removal of Primary Teeth Root Canals: A Scanning Electron Microscopic Study

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Abstract

Introduction: This *in vitro* study was designed to assess the efficacy of 0.9% normal saline, 5.25% Sodium Hypochlorite, MTAD and Ginger extract as final irrigant in the removal of the smear layer in the coronal, middle, and apical thirds of the root canals of primary teeth after hand instrumentation.

Materials and Methods: 28 human extracted primary teeth were prepared to 40 k file and randomly divided into four groups (n=7) 0.9% Normal Saline, 5.25% Sodium Hypochlorite, MTAD and Ginger extract as final irrigant. The decoronated teeth were split longitudinally into two halves and assessed for the amount of smear layer over dentinal surface. SEM images were taken and scoring was done using Rome criteria and data were analysed using Kruskal-Wallis test, chi-square test and student t test.

Results: Intergroup comparison shown statistically significant difference in all thirds with Biopure MTAD ($p<0.0001$) compared with other groups.

Conclusion: Biopure MTAD is an effective solution for the removal of the smear layer and the Ginger extract is an herbal alternative as root canal irrigant in primary teeth.

Keywords: Irrigating solutions; Smear layer; SEM-primary teeth

Introduction

Maintaining the integrity of primary dentition is the most important element in pedodontics. It is virtual to preserve the primary dentition till its normal exfoliation, which is essential for the growth of dentofacial complex [1]. In case of irreversible pulpitis, pulpectomy is a successful meaning to retain the teeth in terms of function, esthetics, arch length, space management and symmetry. In Root canal therapy, biomechanical preparation is a fundamental step in cleaning the root canals but canal ramification in primary tooth makes the its debridement difficult [2]. Therefore, it is imperative to use auxiliary solutions that promote disinfection of these areas, mainly because infected primary teeth can harbor micro-organisms inside the dentinal tubules. The success of endodontic treatment strongly depends on the chemo-mechanical removal of microorganism and pulp debris using instruments in biomechanical preparations and irrigating solutions [3].

During root canal instrumentation smear layer is produced, an amorphous structure composed of inorganic and organic substances covering the dentinal walls of root canals and smear plugs present in depth of dentinal tubules. The inorganic material in the smear layer is composed of tooth structure and some nonspecific inorganic contaminants, while the organic components may consist of heated coagulated proteins, necrotic or viable pulp tissue, and odontoblastic processes plus saliva, blood cells, and microorganism [4]. The removal of the smear layer requires organic and inorganic solvents, which are known as endodontic irrigants. Most commonly used root canal irrigants are normal saline and sodium hypochlorite. Physiological saline has no effect on removing dentinal debris and smear layer [5]. Sodium hypochlorite in 1.0% to 5.25% concentrations has not been shown to effectively remove the smear layer but will dissolve organic tissue. EDTA and citric acid are most commonly used irrigant to remove smear layer because of its chelating action. However, the application of higher concentration of citric acid and EDTA irrigation for more than 1 minute and in volume more than 1 ml has been reported to be associated with dentinal erosion. BioPure MTAD has been introduced to dentistry as a final irrigant for smear layer removal [6].

The several undesirable characteristics of commonly used root canal irrigants are tissue toxicity, risk of emphysema, allergic potential, disagreeable smell and taste, has prompted researchers to look for herbal alternatives such as *Morinda citrifolia*, Neem leaf Extract (A*zadirachta indica*), Triphala and Green tea polyphenols, German chamomile. Hence the use of ginger extract (*Zingiber officinale*) as endodontic irrigant has been used in present study [7].

The aim of this study was to evaluate the efficacy of various irrigating solutions likes 0.9% Normal Saline, 5.25% Sodium Hypochlorite, MTAD and Ginger extract as final irrigants in the removal of the smear layer in the coronal, middle, and apical thirds of the root canals of primary teeth after hand instrumentation [8].

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Materials and Methods

Prior to the commencement of this *in vitro* study, Institutional Ethical Committee (IEC) approval was obtained. The study was done on 28 extracted single rooted deciduous teeth; the samples were divided randomly into four groups (7 teeth in each group) depending on the final irrigant solution as follows: Group I-0.9% Normal Saline (used as a negative control group), Group II-5.25% sodium hypochlorite, Group III-Biopure MTAD, mixture of tetracycline, an acid and detergent (Tween 80), Group IV-alcoholic extract of ginger [9]. Extracted single rooted deciduous teeth were obtained from the patients visiting department of pedodontics and preventive dentistry, hitkarini dental college and hospital Jabalpur MP, India and various Hospitals , tooth were extracted for various reasons like trauma, over retained teeth, extraction for the orthodontic reasons and primary incisors with at least two third of root intact were included in the study. Teeth with abnormal root morphology, internal resorption and those that are endodontically treated were excluded from the study. Extracted human deciduous single rooted teeth were collected, disinfected and stored in 0.9% saline at 4°C till further use and handled as per the recommendation and the guidelines laid down by Center for Disease Control (CDC) [10].

Preparation of ginger extract

Fresh ginger were chopped into small pieces, dipped and washed in distil water. 50 ml of ethylene diethyl ether was added into 20 gm of chopped ginger and was subjected to hot soxhlet extraction for 24 hours cycle at 60°C-65°C. The resulting extract of ginger was concentrated by evaporation of solvent (ethylene di ethyl ether) completely, at under reduced pressure by lyotrap dryer [11].

Specimen preparation

The 28 extracted human deciduous anterior teeth were decoronated at the level of the Cemento-Enamel Junction (CEJ) and superficial grooves were placed mesiodistally along the longitudnal axis in cementum not extending to the root canal, using diamond disk mounted on a low speed hand piece, to facilitate smooth split in the latter stages for scanning electron microscopic examination. Instrumentation was done with separate set of 'K' files for each group 10 K-file was placed in the canal until it was just visible at the apical foramen [12]. The working length was established by reducing 1 mm from this point. Bio-mechanical preparation was performed using step-back technique with file numbers sequencially #15, #20, #25, #30, #35 and #40 in the entire working length of the canal. Between every instrument, irrigation was performed with 3 ml of 0.9% Normal Saline for 10 seconds. The instrument was only exchanged for another of larger diameter when the previous one moved freely inside the canal. In the present study, for irrigation, 26 gauge needles were used and the depth of the irrigation needle was calculated by reducing 2 mm from the working length [13].

Before final irrigation, the apical ends of the root were sealed with sticky wax, to stimulate an *in vivo* apical counter pressure and to prevent extrusion of irrigant through the apical foramen [14]. Following this, on the basis of final irrigation, the samples were divided randomly into four groups as follows:

Group I (0.9% normal saline): 7 teeth were taken in the group and canals were finally irrigated with 5 ml of 0.9% Normal saline for 5 min in combination with manual agitation using #40 gutta percha cone.

Group II (5.25% sodium hypochlorite): 7 teeth were taken in the group. Each root canals was flooded with 1ml of 5.25% Sodium Hypochlorite solution, in combination with manual agitation using #40 gutta percha cone and then slow flushing of remaining 4 ml irrigant, for 5 min.

Group III (Biopure MTAD, mixture of tetracycline, an acid, citric acid and detergent, tween 80): 7 teeth were taken in the group. Each root canals was then flooded with 1ml of Biopure MTAD, in combination with manual agitation using #40 gutta percha cone and then slow flushing of remaining 4 ml irrigant, for 5 min.

Group IV (alcoholic extract of Ginger): 7 teeth were taken in the group. Each root canals was flooded with 1ml of Ginger Extract, in combination with manual agitation using #40 gutta percha cone and then slow flushing of remaining 4 ml irrigant, for 5 min.

The solution is agitated with the help of #40 gutta percha to facilitate the solution all over the canal surface. All the samples were finally irrigated with 10 ml of distilled water to avoid sedimentation of crystals within the canals (except for Biopure MTAD) and dried with absorbent paper point [15].

Specimen sections

The teeth were split longitudinally, with the aid of a BP knife (blade #15) and a surgical mallet, through the previously placed grooves and half of each tooth was placed in a 2% glutaraldehyde solution for 24 h. The other half of each tooth was discarded. Then the samples were transferred to the testing lab in a sterilized plastic container without any contamination for scanning electron microscopic analysis. The tooth were dehydrated with ascending concentrations of ethyl alcohol (70%, 90%, 95% and twice at 100%) and placed in a desiccator for at least 24 h [16].

Examination under scanning electron microscope

The dried specimens were, mounted on metallic stubs, sputter coated with~35 nm layer of gold-palladium particles, to render a conductive surface, and evaluated under a scanning electron microscope at magnifications of 2,000X at the coronal third, middle third and apical third of the dentinal surface. A standardized series of photographs at a 2000X magnification was obtained at coronal, middle, apical third for comparative purpose [17]. The scanning electron microscopic images were analyzed according to rating criteria system developed a score of 0-No smear layer, all dentinal tubules open and no erosion of tubules, 1-No smear layer, all dentinal tubules open and erosion of tubules, 2-Minimum smear layer>50% dentinal tubules visible. 3-Moderate smear layer<50% of dentinal tubules open and 4-Heavy smear layer; outline of dentinal tubules obliterated [18].

In vitro

Results

The pictures from the scanning electron microscopy showed that

among the tested irrigants Scores attributed to each specimen in the three thirds of the canals and using SPSS software, the data obtained were subjected to statistical analysis using Kruskal-Wallis test, chi-square test and student T test [19]. The ginger extract (Group IV) has significantly less mean score (Table 1) with respect to all thirds when compared to normal saline (Group I) and 5.25% NaOCl (Group II) but significantly more when compared to biopure MTAD (Group III) (Figure 1) [20].

Table 1: Mean value scores and standard deviation of the groups at coronal, middle and apical third.

Groups	Coronal third	Middle third	Apical third
Group I (normal saline)	3.86 ± 0.378	4.00 ± 0.000	4.00 ± 0.000
Group II (5.25% sodium hypochlorite)	3.43 ± 0.787	3.71 ± 0.488	3.86 ± 0.378
Group III (Biopure MTAD)	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Group IV (ginger extract)	2.43 ± 0.787	2.14 ± 1.215	2.86 ± 0.690

In coronal third the mean rank biopure MTAD was significantly lower from each respective groups and statistically it was highly significant ($\chi2$=21.287; p<0.0001). The lower ranks of smear layer removal efficacy confirms the accuracy of the biopure MTAD followed by ginger extract then sodium hypochlorite and least for normal saline. At middle third the mean rank of smear layer removal were found as 22.00 for normal saline, 19.71 for 5.25% Sodium hypochlorite, 4.50 for biopure MTAD and 11.79 for ginger extract. The mean rank at middle third was also lowest for biopure MTAD and statistically it was highly significant ($\chi2$=22.595; p<0.0001 (Table 2) [21].

At apical third the mean ranks was 21.50 for Normal saline, 20.14 for 5.25% sodium hypochlorite, 4.00 for biopure MTAD and 12.36 for Ginger extract. Biopure MTAD produced the lowest rank of score and statistically it was highly significant ($\chi2$=22.693; p<0.0001) (Table 3) [22].

Comparison between group III (biopure MTAD) with all other groups are highly significant result (p<0.0001) followed by group IV (ginger extract) followed by group II (5.25% sodium hypochlorite) and then Group I (normal saline) (Figures 2-13) [23].

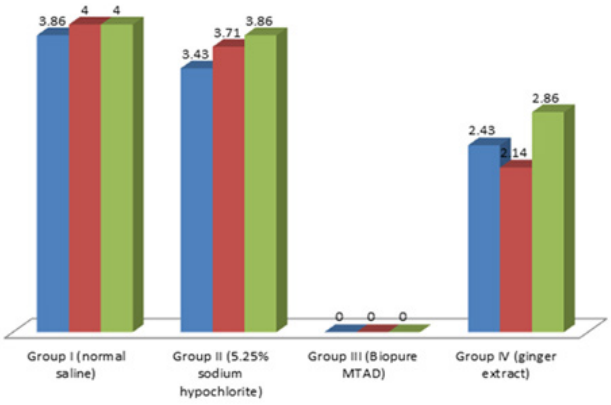

Figure 1. Mean value scores of the groups at coronal, middle and apical third. **Note:** (■) Coronal third; (■) Middle third (■) Apical third.

Table 2: Mean rank of all experimental group at respective third using kruskal-wallis test and Chi-Square test.

	Group	N	Mean rank	Chi-sqaure test	Df	Asymp. sig
Coronal third	Normal saline (Group I)	7	21.93	21.387	3	0.0001
	5.25% sodium hypochlorite (Group II)	7	19.07			
	Biopure MTAD (Group III)	7	4			
	Ginger extract (Group IV)	7	13			
Middle third	Normal saline (Group I)	7	22	22.595	3	0.0001
	5.25% sodium hypochlorite (Group II)	7	19.71			
	Biopure MTAD (Group III)	7	4.5			
	Ginger extract (Group IV)	7	11.79			
Apical third	Normal saline (Group I)	7	21.5	23.693	3	0.0001
	5.25% sodium hypochlorite (Group II)	7	20.14			
	Biopure MTAD (Group III)	7	4			
	Ginger extract (Group IV)	7	12.36			

Table 3: Overall intercomparison of all four groups using student T test.		
Comparison of groups	**T**	**P**
Group I *vs.* Group II	2.12	p>0.05
Group I *vs.* Group III	83	p<0.0001
Group I *vs.* Group IV	5.19	p<0.001
Group II *vs.* Group III	29.1	p<0.0001
Group II *vs.* Group IV	3.87	p<0.05

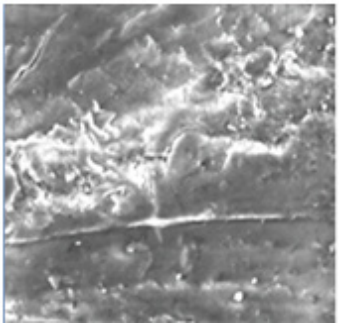

Figure 2. Scanning Electron Microscopy (SEM) picture of group I (normal saline) coronal third SEM picture of the root canal wall of sample in group I.

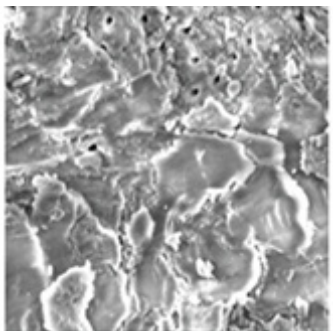

Figure 3. Scanning Electron Microscopy (SEM) picture of group I (normal saline) middle third SEM picture of the root canal wall of sample in group I.

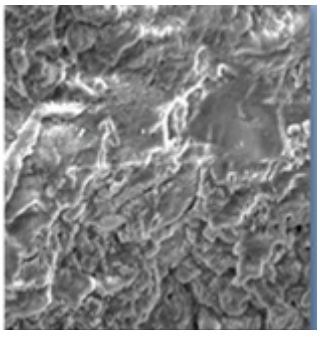

Figure 4. Scanning Electron Microscopy (SEM) picture of group I (normal saline) apical third SEM picture of the root canal wall of sample in group I.

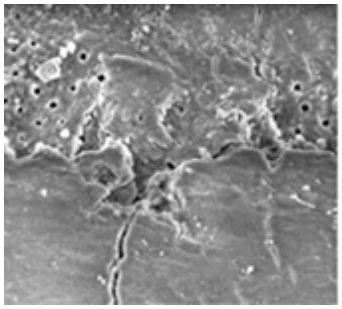

Figure 5. Scanning Electron Microscopy (SEM) picture of group II (5.25% sodium hypochlorite) coronal third SEM picture of the root canal wall of sample in group II.

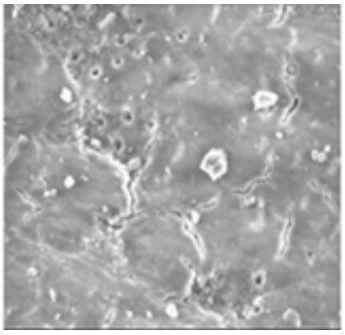

Figure 6. Scanning Electron Microscopy (SEM) picture of group II (5.25% sodium hypochlorite) middle third SEM picture of the root canal wall of sample in group II.

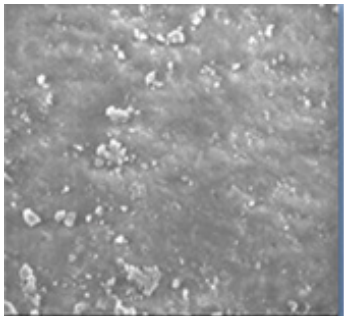

Figure 7. Scanning Electron Microscopy (SEM) picture of group II (5.25% sodium hypochlorite) apical third SEM picture of the root canal wall of sample in group II.

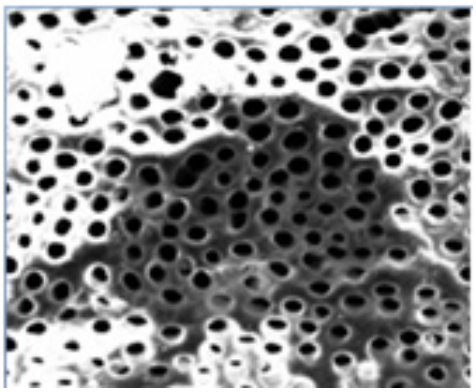

Figure 8. Scanning Electron Microscopy (SEM) picture of group III (biopure MTAD) coronal third SEM picture of the root canal wall of sample in group III.

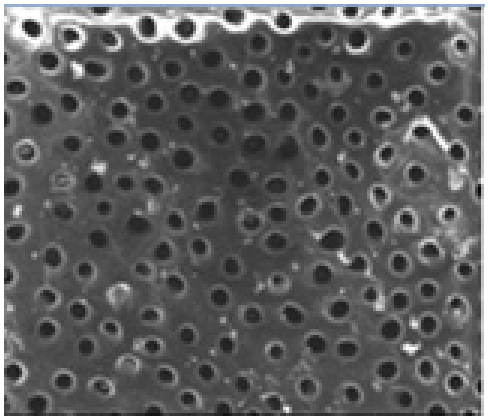

Figure 9. Scanning electron microscopy (SEM) picture of group III (biopure MTAD) middle third SEM picture of the root canal wall of sample in group III.

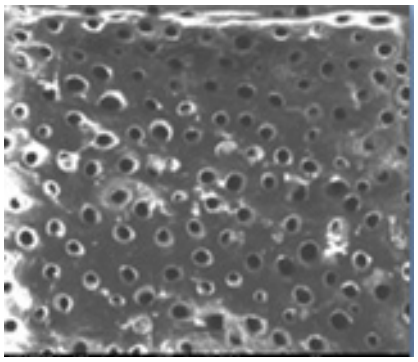

Figure 10. Scanning electron microscopy (SEM) picture of group III (biopure MTAD) apical third SEM picture of the root canal wall of sample in group III.

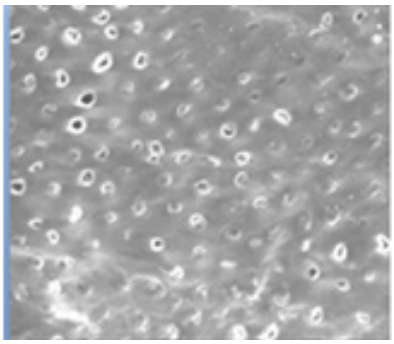

Figure 11. Scanning electron microscopy (SEM) picture of group IV (ginger extract) coronal third SEM picture of the root canal wall of sample in group IV.

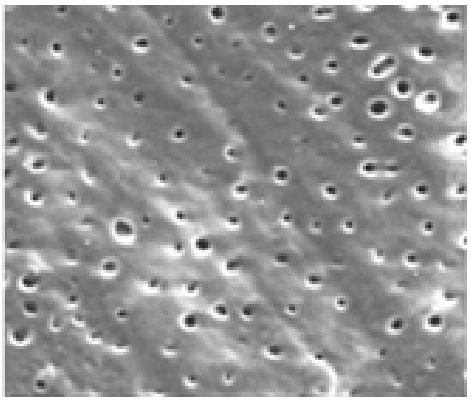

Figure 12. Scanning Electron Microscopy (SEM) picture of group IV (ginger extract) middle third SEM picture of the root canal wall of sample in group IV.

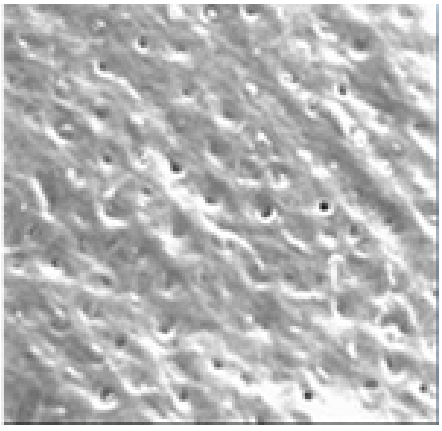

Figure 13. Scanning Electron Microscopy (SEM) picture of group IV (ginger extract) apical third SEM picture of the root canal wall of sample in group IV.

Discussion

Current endodontic instrumentation methods produce a smear layer that covers the root canal surfaces. The smear layer contains inorganic and organic substances, which include fragments of odontoblastic processes, microorganisms, their by-products, and necrotic materials. The smear layer is made of particles ranging in size from less than 0.5 µm-15 µm. Smear layer, being a loosely adherent structure, should be completely removed from the surface of the root canal wall because it can harbour bacteria and provide an avenue for leakage [24]. It may also limit the effective disinfection of dentinal tubules by preventing sodium hypochlorite, calcium hydroxide and other intracanal medicaments from penetrating the dentinal tubules. Because of potential contamination and adverse effect of the smear layer on the outcome of root canal therapy, it seems reasonable to suggest removal of the smear layer for disinfection of the entire root canal system [25].

Current methods of smear layer removal include chemical, ultrasonic, and laser techniques-none of which are totally effective or have received universal acceptance. The importance of irrigation as a method of removing smear layer from the root canal is well recognized because it has been shown that when instrumentation was carried out without irrigation, 70% debris remained in the root canal. Smear layer could lead to loss of working length, hindered penetration of the intracanal medicaments, sealants and increases the chances of microleakage and forcing the debris into the periapical region. The efficiency of the smear layer removal is related to both, the type of the irrigant used and the method of flushing [26].

There are number of studies reported in the literature explaining the smear layer removal in permanent root canals and conclusive guidelines are outlined for the smear layer removal in permanent teeth. But literature available regarding smear layer removal of primary teeth is scarce. However, only limited studies have been done regarding removal of smear layer in primary teeth. Thus the present study was conducted to assess the efficacy of 0.9% normal saline, 5.25% sodium hypochlorite, biopure MTAD and ginger extract as final irrigant in the removal of the smear layer in the coronal, middle, and apical thirds of the root canals of primary teeth after hand instrumentation [27].

Sodium hypochlorite used in this study, is a weak alkaline/ base that acts on the albumin (remains of pulpal tissue, foods and microorganisms), denaturing them and turning them soluble in water. Like soap, it facilitates the removal of debris from the root canals and, in spite of being a necrosis agent (to act on organic matter) it is little poisonous or irritating to the live tissues. The NaOCl alkali contacting with organic products in decomposition liberates chlorine and nascent oxygen that promote bactericidal action.

Biopure MTAD a new irrigating solution containing 3% doxycycline hyclate, 4.25% citric acid and 0.5% Polysorbate 80 (Tween 80) detergent, represents an innovative approach in simultaneous removal of the endodontic smear layer and disinfection of root canals. They said that MTAD does not significantly change the structure of the dentinal tubules when canals are irrigated with sodium hypochlorite followed by final rinse of biopure MTAD. Studies on the use of this effective irrigant, biopure MTAD on deciduous teeth are still required and are in progress.

Ginger extract was chosen as an experimental group in this study. History of ginger and its applications were well documented, Ginger (*Zingiber officinale*, F. *Zingiberaceae*) has been listed as "Generally Recognized as Safe" (GRAS) document in Food Drug Administration. Its healing ability, antinflammatory and antimicrobial activity is well documented in endodontics, but still further investigation for other properties are required.

Scanning electron microscopic pictures of 5.25% Sodium hypochlorite (group II) demonstrated the absence of superficial debris but presence of smear layer. Under high magnification (× 2000), the presence of smear layer was noticed at all three root thirds (coronal, middle, apical) in 71% of sections. Even though some samples showed the removal of smear layer partially in 29% of sections, it was clearly understood that the ability to remove the smear layer was incomplete. The smear layer removal was quite better in coronal third compared to middle and apical third. The scores of the sodium hypochlorite group shown that its efficacy was clearly better than that of the Normal saline (Group I). But it is proved that 5.25% Sodium hypochlorite is very much effective in removing the organic debris. It can be concluded that even though sodium hypochlorite partially removes the smear layer, it is incomplete and not acceptable.

The experimental irrigant biopure MTAD (group III) is now commercialized as biopuretm MTAD. For optimal removal of the endodontic smear layer and to avoid inadvertent erosion of the intra-radicular dentine, a revised protocol had been proposed which involves the use of an initial rinse with 1.3% NaOCl followed by the use of MTAD as the final rinse for a cumulated period of 5 min. In the present study only MTAD was used without NaOCl rinse as, it has been recently reported in an *in vitro* study that intrinsic staining of dentine occurred after natural light exposure of the NaOCl and MTAD-irrigated coronal and intra-radicular dentine and reduction in antimicrobial substantivity of MTAD after initial sodium hypochlorite irrigation. A possibility is the reaction between biopure MTAD and sodium hypochlorite which resulted in the formation of a brown solution which was also reported, in the absence of light exposure, when biopure MTAD was employed as the initial rinse followed by the use of different concentrations of sodium hypochlorite as final rinse. Bench top reproduction of the phenomenon revealed that the redox reaction between sodium hypochlorite and biopure MTAD resembled the mechanism of tetracycline tooth staining. However this phenomenon may be prevented by treating the sodium hypochlorite-irrigated dentine with Ascorbic Acid, an anti-oxidant, before the application of MTAD.

Scanning electron microscopic pictures of biopure MTAD (group III) demonstrated the absence of smear layer in and around dentinal tubules and whole dentinal surface was free of smear layer in 100% of sections in all the thirds. We found that agitation of MTAD resulted in better removal of the smear layer and debris specially in apical third. This was probably due to adequate penetration of the solution into the apical portion of the canal during irrigation. There are studies reported in literature which shows biopure MTAD to be a superior

antimicrobial solution and showed its superior antimicrobial activity compared with Sodium Hypochlorite or EDTA when tested against Enterococcus faecalis. 3% doxycycline hyclate, a tetracycline isomer present in biopure MTAD act as both chelating agent as well as antimicrobial agent.

Ginger root is the rhizome of the plant Zingiber officinale, a herb that is used as a spice and also for its therapeutic qualities. The pungent substances namely gingerol and shagelol were identified as more active agents. Other than these compounds Ginger rhizome also contain, zingerone, paradol and volatile oil. The volatile oil consists of mainly mono and sesquiterpenes; camphene, beta-phellandrene, curcumene, cineole, geranyl acetate, folic acid, terphineol, terpenes, borneol, geraniol, limonene, linalool, alpha-zingiberene (30%-70%), beta-sesquiphellandrene (15%-20%), beta-bisabolene (10%-15%) and alpha-farmesene, in addition to the oleoresin zingiberol, the principal aroma contributing component as well as zingiberene, gingediol, diarylheptanoids and phytosterols. In the present study scanning electron microscopic pictures of ginger extract (group IV) demonstrated that 57% sections were shown more than 50% dentinal tubules open, 23% sections shown less than 50% dentinal tubules, 14% specimen shown specimen fully covered by smear layer and only 5% specimen shown complete removal of smear layer, which shows that ginger extract was able to remove the debris, and organic part and it was partially able to remove the smear layer. The smear layer removal was more in the middle third than coronal third and least at the apical third.

The overall results of the present study, confirmed that a heavy smear layer was observed at all levels in the specimens irrigated with Normal saline solution, consistent with previously published reports, which found that saline alone produces a sludge layer made up of residual debris that occluded the dentinal tubules. 5.25% Sodium Hypochlorite removes the organic tissue but not able to remove the smear layer proved in various studies, but due to its high antimicrobial property and its cost effectiveness it can be suggested to use this solution combined with biocompatible chelating agent in primary teeth. MTAD, when used alone with manual agitation, leads to effective removal of the smear layer in all thirds of the root canal of primary tooth, when used for recommended time (5 min). Based on this, it can be suggested to use MTAD with manual agitation in primary teeth. It is interesting to see the results of ginger extract (group IV), where there was partial smear layer removal which was superior to 5.25% Sodium Hypoclorite (group II), but less effective than biopure MTAD (group III). With its antimicrobial effectiveness proved in various studies, it can be introduced in pediatric dentistry as a newer root canal irrigant in horizon [28].

Conclusion

Based on the results of the present study it can be concluded that biopure MTAD is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules. The study suggest use of biopure MTAD, an innovative solution and the ginger extract as an herbal alternative in primary teeth, but within the limitation of the present study further *in vitro* and *in vivo* studies will be required to determine their effectiveness.

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