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ROOT CANAL IRRIGANT COMBINING SALVADORA PERSICA WITH SODIUM HYPOCHLORITE – ANTIMICROBIAL, TISSUE DISSOLUTION ,CHELATING ACTION & CHANGES IN CA/P OF ROOT DENTIN :IN VITRO STUDY

Endodontic

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ABSTRACT

OBJECTIVE: Currently none of the irrigants used in endodontic therapy tested so far either alone or in combination could satisfy all criteria of ideal irrigant. Search for such a single agent which is more biocompatible along with other properties of irrigant continues. This study is aimed to evaluate pulp dissolution, antimicrobial activity, smear layer removing ability, and change in the Ca/P ratio of root dentin by using experimental formulation combining 3 % & 5 % Sodium hypochlorite with 40% Salvadora Persica & comparing it with 3 % Sodium Hypochlorite & 17%EDTA **Materials and Methods:Pulp dissolution:** Human pulp tissue weighing 0.9g was dissolved in each experimental group G1:3%NaOCl,G2:3%NaOCl+40% S.persica, G3: 5%NaOCl +40% S.persica and weighed after the passage time of 10 min , 20min , 30 min. The difference in weight of the tissue sample before and after exposure to the test solution was assessed. **Antibacterial activity:** Antimicrobial efficacy of 3% NaOCl, 3% NaOCl + 40% S.Persica,5%NaOCl + 40% S.persica as experimental group against E.fecalis was assessed using direct contact test. Statistical Analysis for both the test was carried out using Kruskal Wallis and Post Hoc Mann Whitney Analysis Smear layer removal& Ca/p ratio analysis: Single rooted premolars were divided in 3 groups (n=33) and irrigated with G1: 17% EDTA , 3% NaOCl, G2 : 3% NaOCl+ 40% S. persica, G3: 5% NaOCl + 40% S.persica. Each tooth was then split longitudinally. One half of sample from each group were subjected to scanning electron microscope to assess smear layer removal and second half for EDX analysis to check Ca/P ratio. **Statistical Analysis:** SEM – Kruskal Wallis and Post Hoc Mann Whitney EDX – ANOVA and Tukey'S HSD Post hoc Analysis

RESULTS:

- Pulp dissolution by freshly mixed G3 was superior to G1 & G2. There is no difference in pulp dissolution efficacy of G1 & G2.
- No statistically significant difference was seen with respect to smear layer removal among the groups tested. EDX analysis showed significantly low Ca/P ratio for G1 compared to other groups and no statistically significant difference was seen b/w G2 &G3
- In both the time interval tested (5 min, 10min) G3 showed maximum antimicrobial efficacy followed by G1 and G2 in terms of mean value.

CONCLUSION: The combined formulation of 5% NaOCl + 40 % S.Persica was most effective root canal irrigant among the groups tested in terms of pulp dissolution, antimicrobial &chelating action. 3% NaOCl + 40% Salvador persica was found to be as effective as 3% NaOCl and 17% EDTA. Experimental irrigants were found to be less erosive to root dentin in both combinations tested.

KEYWORDS

EDTA. Sodium hypochlorite, Salvadora perisca, In Vitro Study

INTRODUCTION

An ideal endodontic irrigant is one which possess tissue dissolution property, antimicrobial activity, smear layer removing ability while being compatible with root dentin. Unfortunately, none of the currently available irrigants can be used alone to eliminate both organic& inorganic content of root canal space simultaneously.

Literature describes a variety of chemicals with a broad range of concentrations and different irrigation regimens to achieve the various desirable actions of the irrigant. However, use of multiple irrigants is time consuming and also increases clinician's fatigue.

The most commonly used irrigant is Sodium hypochlorite (NaOCl) in concentrations ranging from 1 - 5.25%1. The preference for this chemical over other irrigants is due to its unique ability to dissolve pulp tissue, and its excellent antimicrobial potency2. Limitations of NaOCl include its inability to remove the smear layer by itself. Thus, it has to be used in conjunction with 17% EDTA3.

Grawehr et al in a study combined NaOCl with EDTA, it resulted in reduced efficacy of NaOCl due to reduction in chlorine content4. Also, Baumgartner et al showed increased dentinal erosion when sodium hypochlorite was combined with EDTA. Apparent aggressiveness of EDTA in causing erosion of radicular dentine is attributed to the prolonged initial contact of the dentine by NaOCl rendering the root-treated teeth more prone to fracture5.

Brian j rasimick et combined 2 % & 20% Chlorhexidine with 17% EDTA which resulted in formation of white precipitate (salt) due to the electrostatic neutralization of the cationic chlorhexidine by the anionic EDTA6.

So since none of the irrigants tested so far either alone or in combination could satisfy criteria of ideal irrigant, search for such a single agent which is more biocompatible along with other properties of irrigant continues.

In recent years, herbs have been tried as irrigants due to their high antimicrobial activity, biocompatibility, anti-inflammatory and antioxidant properties7. The major advantages of herbal irrigants are low tissue toxicity, easy availability, cost effectiveness and limited microbial resistance8. Some preliminary studies have been conducted using certain herbs for root canal irrigation that have shown encouraging results.

Salvadora persica or pilu (S. persica), a commonly used agent in many herbal mouth rinse and toothpaste has got many properties like antimicrobial, anti-cariogenic, antioxidant and chelation actions. It has exhibited a potent antimicrobial activity against many organisms including E. faecalis 9. The ability of S. persica to remove the smear layer may be attributed to its acid content (stearic acid), which may react with calcium in the dentin and act as a chelating agent[10].

A Study done by Hanan Balto et al has shown that 5mg/ml S. persica solution was as effective as 17% EDTA in removing the smear layer from the coronal third, middle third of the canal wall10.

Salvadora has shown to be antimicrobial and possess smear layer removing ability while being more biocompatible than EDTA9,10. But it lacks tissue dissolving property.

Therefore, an experimental formulation combining Sodium hypochlorite and Salvadora persica, will be investigated as root canal irrigant for tissue dissolution property, antibacterial efficiency, and

smear layer removing ability.

MATERIALS AND METHODOLOGY

PULP DISSOLUTION:

Groups: (n=33)
G1: 3% NaOCl,
G2: 3% NaOCl + 40% S. persica (6: 4),
G3: 5% NaOCl + 40% S. persica (6: 4)

Pulp tissue were collected and sectioned into pieces using surgical blade to weight 0.9 mg (measured using precision balance in air tight container).The pulp tissue was then immersed into test solutions and reweighed after passage time of 10 min ,20min, 30 min. The difference in weight of the tissue sample before and after exposure to the test solution was divided by the original weight and multiplied by 100 to obtain the percentage of tissue weight loss. The values were analyzed statistically by using Kruskal Wallis and Post Hoc Mann Witney analysis. (Sodium hypochlorite (Deor), 40% Salvadora persica (Himalaya Drug Company, Bangalore).

SMEAR LAYER REMOVAL AND EFFECT ON Ca/P RATIO OF ROOT DENTIN

(n=33)
G1: Irrigation with 5 ml 17% EDTA followed by 3% NaOCl for 1 min
G2: Irrigation with 5 ml of 3% NaOCl + 40% S. persica for 1 min
G3: Irrigation with 5 ml of 5%NaOCl + 40% S. persica for 1 min

Freshly extracted single rooted premolars. Teeth were decoronated to a standard length of 15mm with a diamond disc. Patency of apical foramen was established by inserting a size 15 K file till it was visible at the apical foramen and the working length 1 mm short from this length was established .The root canals of all the groups were enlarged till Protaper rotary instrument F3 (Dentsply Sirona).

The irrigating solutions were delivered via a sterile 30-gauge needle. The root canals were then flushed with 10 ml of distilled water dried with sterile paper points. Longitudinal grooves were made on the bucco-lingual surfaces on each root by using a diamond disk at low speed without penetrating the canal, the roots were then split in two halves with a chisel. For each root, the half containing the most visible part of the apex and best represented the total canal length was selected. The samples further divided into coronal, middle and apical third. The samples were subjected to EDX analysis to determine the alteration in Ca/P ratio caused by the various irrigant and for SEM analysis(ESEM Quanta 200, FEI) to determine the amount of dentinal tubule opening. Digital photograph was taken at 2000 X.

Analysis of the SEM images was performed to score the presence of smear layer on the surface of the root canal in the coronal, middle and apical portion of each canal based on the criteria described by Hulsmann et al .[11](2002) outlined below :

Score	Description
1	No smear layer and all dentinal tubules open
2	A small amount of smear layer and some dentinal tubules open
3	Homogenous smear layer covering root canal wall and only a few dentinal tubules open
4	Complete root canal wall covered by homogeneous smear layer and no open dentinal tubules
5	Heavy homogeneous smear layer covering the complete root canal wall

The obtained values were analyzed statistically by using Kruskal Wallis and Post Hoc Mann Witney analysis.
Antimicrobial Study

(n=33)
G1: 3% NaOCl and 17%EDTA,
G2: 3% NaOCl + 40% S.persica(6 : 4)
G3: 5% NaOCl + 40% S.persica(6 : 4)

Single rooted human mandibular premolars with fully formed apices was chosen for the study. All the teeth were decoronated at CEJ, with a diamond disc to obtain a standard tooth length of 15 mm. A size 15 K(MANI Japan) file was used to scout and establish patency till the tip of the file was seen at the apical foramen, from which 1 mm was subtracted to establish the working length. The samples were then instrumented with pro taper rotary system upto size F3 (DENTSPLY SIRONA) to allow for adequate flushing and penetration of irrigant. 2ml of 3%Naocl was used as a working solution during instrumentation. teeth apices sealed with double coat of nail polish.

The roots were then sterilized in an autoclave at 1210C and 15lb pressure. A 24hr culture of E. faecalis ATCC 29212 was grown in brain heart infusion broth with concentration of 1x1cells/ml. Each root canal was then inoculated with 10µl of E. faecalis suspension using a sterile 1ml tuberculin syringe and incubated at 37 for 48 hrs. 100µl of test solution was then added to each canal for 5 &10 mins and then was diluted with saline to prevent any carryover of irrigants. It was then transferred onto BHI agar plates & incubated at 370C for 48hrs. Colonies were counted after 48hr of incubation at 370C and expressed as colony forming units (CFU's) per millilitre. The antimicrobial effect of experimental formulation was then recorded at 5 & 10 mins. The number of Colony Forming Units (CFU's) of E.faecalis served as a measure of antibacterial activity.

RESULTS

TABLE 1: COMPARISON OF MEAN PULP TISSUE DISSOLVING POTENTIAL OF 3 STUDY GROUPS TEST AT DIFFERENT TIME INTERVALS USING KRUSKAL WALLIS TEST

TIME PERIOD	3% NaOCl	Std. Deviation	3% NaOCl + 40% S.persica	Std. Deviation	5% NaOCl + 40% S.persica	Std. Deviation	P value
10 min	4.35	.582	4.60	.449	5.80	.610	0.00*
20 min	1.05	.336	1.35	.354	2.80	.385	0.00*
30 min	3.60	0.737	3.05	0.367	0.35	.219	0.00*

*Significant P<0.05

TABLE 2: COMPARISON OF PULP DISSOLUTION AMONG GROUPS AT 10, 20 AND 30 MIN USING POST-HOC MANN-WHITNEY

GROUPS	10 min		20 min		30 min	
	Mean difference	P value	Mean difference	P value	Mean difference	P value
Group 1 * Group 2	-0.25	0.30	-0.30	0.052	0.55	0.08
Group 1 * Group 3	-1.45	0.00*	-1.75	0.00*	3.25	0.00*
Group 2 * Group 3	-1.20	0.00*	-1.45	0.00*	2.7	0.00*

*Significant P<0.05

- From (Table 1 & 2) 5.25% NaOCl + 40% S.persica at all tested time intervals (10 min,20 min,30 min) showed significantly more pulp tissue dissolution compared to 3% NaOCl and 3% NaOCl + 40% S.persica.
- No significant difference in pulp dissolution efficacy of 3% NaOCl & 3% NaOCl + 40% S.persica

GRAPH 1: MEAN PULP DISSOLUTION VALUE

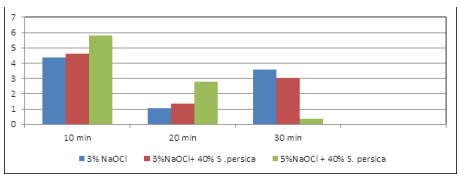

TABLE 3: COMPARISON OF MEAN SCORE FOR SMEAR LAYER REMOVAL BETWEEN 3 STUDY GROUPS AT CORONAL MIDDLE AND APICAL THIRD OF ROOT CANAL USING KRUSKAL WALLIS TEST

REGION	3%NaOCl ,17%EDTA	Std. Deviation	3%NaOCl+ 40%S.persica	Std. Deviation	5%NaOCl + 40%S.persica	Std. Deviation	P Value
Coronal Third	1.85	.795	1.79	.740	1.79	.781	0.94

Middle Third	1.79	.600	1.70	.770	1.67	.777	0.58
Apical Third	3.45	.506	3.42	.502	3.39	.556	0.92

*Significant <0.05

- No statistically significant difference was seen with respect to smear layer removal among the groups tested (Table 3)

GRAPH 2: MEAN VALUE GRAPH FOR SEM

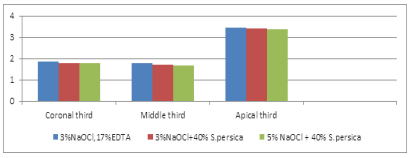

FIGURE 1: SEM IMAGES

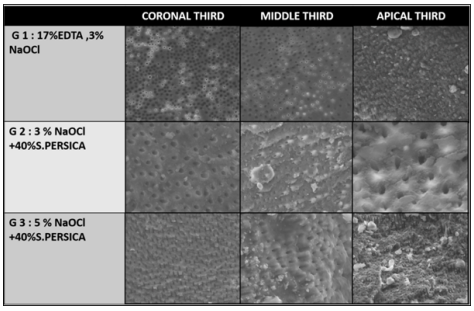

TABLE 4: COMPARISON OF MEAN CA/P RATIO OF ROOT DENTIN BETWEEN 3 STUDY GROUPS IN CORONAL MIDDLE AND APICAL THIRD OF ROOT CANALUSING ONE WAY ANOVA TEST

	3%NaOCl ,17%EDTA	Std. Deviation	3%NaOCl+ 40%S.persica	Std. Deviation	5%NaOCl + 40%S. persica	Std. Deviation	P Value
CORONAL THIRD	1.750	0.450	2.600	0.587	2.430	0.548	<0.001 *
MIDDLE THIRD	1.900	0.508	2.620	0.471	2.480	0.462	<0.001 *
APICAL THIRD	2.000	0.518	2.981	0.632	2.700	0.571	<0.001 *

P<0.001 Significant *

TABLE 5: MULTIPLE COMPARISON OF MEAN DIFFERENCE CA/P RATIO BETWEEN 3 GROUPS AT DIFFERENT REGIONS USING TUKEY'S HSD POST HOC ANALYSIS

Regions	(I) Groups	(J) Groups	Mean Diff.(I-J)	P-Value
Coronal	Group 1	Group 2	-0.850	<0.001*
		Group 3	-0.680	<0.001*
	Group 2	Group 3	0.170	0.40
Middle	Group 1	Group 2	-0.720	<0.001*
		Group 3	-0.580	<0.001*
	Group 2	Group 3	0.140	0.47
Apical	Group 1	Group 2	-0.981	<0.001*
		Group 3	-0.700	<0.001*
	Group 2	Group 3	0.281	0.12

- There was a statistically significant difference in the mean Ca/P ratio between 3 study groups at P<0.001.
- Multiple comparisons between the groups using Tukey's HSD post hoc analysis revealed that group 1 presented a statistically significant lowest mean score compared to group 2 & group 3, both at P<0.001 respectively. However, the mean score between group 2 & group 3 did not present a statistically significant difference. Similar results were found for middle and apical third of the root

GRAPH 3: MEAN VALUE GRAPH Ca/P RATIO

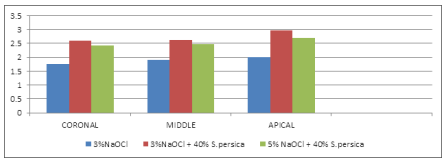

TABLE 6 : COMPARISON OF THE GROUPS FOR ANTIBACTERIAL EFFICACY USING KRUSKAL- WALLIS AT 5 MIN

TIME PERIOD	3% NaOCl	Std. Deviation	3% NaOCl + 40%S.persica	Std. Deviation	5% NaOCl + 40%S.persica	Std. Deviation	P value
5 min	7100.00	1171.89	6500.00	2225.30	7900	5271.32	0.65
10 min	3	1.506	2	.966	4	2.129	0.012

*Significant P<0.05

TABLE 7: COMPARISON OF 3 STUDY GROUPS USING POST- MANN WHITNEY AT 5 MIN & 10 MIN

GROUPS	5 min		10 min	
	Mean difference	P value	Mean difference	P value
Group 1 * Group 2	-800.0	0.67	-1.00	0.18
Group 1 * Group 3	600.0	0.29	1.00	0.05*
Group 2 * Group 3	1400	0.59	2.00	0.005*

*Significant P<0.05

From Table 6 & Table 7:

- In both the time interval tested (5 min, 10min) 5% NaOCl + 40% S. perisca showed maximum antimicrobial efficacy followed by 3% NaOCl and 3% NaOCl + 40% S. persica in terms of mean value.
- However, at 5 min interval, no statistically significant difference was seen among the groups tested while at 10 min, Statistically significant higher antimicrobial efficacy was seen with 5% NaOCl+ 40 S.persica when compared to 3% NaOCl + 40 % S. persica and 3% NaOCl .

DISCUSSION

Irrigants play a very critical role in success of endodontic therapy as it aids in cleaning of the root canal space by flushing out debris, antimicrobial effect, provide tissue dissolution and also eliminates smear layer. As there is no single irrigant that fulfills all these properties combination of irrigants has been tried.

Herbs are being researched extensively in recent years.

Salvadora persica or pilu, is one such herbal irrigant. Studies done by Nawal A.K. Al-sabawi et al12 ,Almas K et al13,and Fahimehsadat Tabatabaei et al14 has shown it to have excellent antimicrobial activity, chelating action while also being biocompatible. But it lacks tissue dissolving property.

Therefore, in this study we tried combining Sodium hypochlorite with Salvadora persica to achieve the combined properties of both the irrigants i.e, pulp dissolution, antimicrobial activity and smear layer removing ability.

On the basis of our pilot study, we combined NaOCl with S.persica in the ratio of 6:4 as the individual properties of these irrigants were not hampered in this ratio.

The dissolving capacity of any irrigant depends on the amount of irrigant, type and contact time with the tissues, area, and mass of the organic tissue 15,16. Therefore, in the present study, these factors were standardized using the same volume of solutions and pulp tissue of

approximately the same shape and weight (0.9 mg). Human pulp tissue was used in this study as it more clinically relevant. The pulp tissue was weighed using Precision scale as more accurately measures the exact dissolution of the tissue.

Results of Present study revealed that 5.25% NaOCl + 40% S.persica at all tested time intervals (10 min,20 min,30 min) showed significantly more pulp tissue dissolution compared to 3% NaOCl & 3% NaOCl + 40% S.persica.

It might be because of higher concentration of NaOCl used. Higher the concentration of NaOCl higher is the amount of chlorine content & better is the tissue dissolving property. This is in agreement with Rosenfeld who reported that 5% NaOCl as an effective solvent of human pulp tissue[17].

Also there was no significant difference in pulp dissolution efficacy of group 1 (3% NaOCl) & group 2 (3% NaOCl + 40% that addition of S. persica has not interfered with pulp dissolution property of NaOCl.

In this study SEM analysis was done to check for smear layer removal by experimental formulation combining S.persica with NaOCl where S.persica was the main chelating agent.

Studies have used S.persica with the concentration of 50%, 25%, 20%, 15% and 4% as a smear layer removal irrigant18.So,40 % concentration of S.persica was used in this study to achieve maximum chelating action. To make valid comparisons among the three irrigants, all the three groups were irrigated with 5 ml of irrigant for 1 min respectively.

On executing SEM analysis on coronal, middle and apical third although there was difference in mean score. G1 when compared to G2 and G3 showed better smear layer removal at all the 3 levels. G2 and G3 showed comparable mean scores at all the 3 levels. But on multiple comparison between the groups using post hoc Mann whitney demonstrated no statistically significant difference found between 3 groups at all three levels.

Stearic acid content of S.persica reacts with calcium in dentin and bring about chelating action, also weak hydrogen bonds that link alcohol to collagen of smear layer can be easily broken leading to separation of smear layer from dentinal surface and exposing the tubules10.

This result is in agreement with Hanan balto et al who reported smear layer removal activity of S. persica and showed comparable results with 17% EDTA at coronal, middle of canal[10].

When coronal third, middle third and apical third were compared for all the groups, it was observed that maximum removal was with coronal third followed by middle third and minimal removal was with apical third. It is because of larger size of the canals in coronal and middle third compared to apical which allowed improved circulation and action of irrigating solution making smear layer removal possible19,20,21.

Energy dispersive x-ray (EDX) analysis is a microanalytical technique that is used to estimate quantitatively the amounts of mineral in a given tooth sample22.So, in order to check for the alteration in dentin surface by Experimental formulation, EDX analysis was conducted.

In this study, results for calcium phosphate ratio was significantly less for G1: 17% EDTA , 3% NaOCl when compared to other two groups. Also, there is no statistically significant difference b/w G2: 3% NaOCl + 40% S.persica, G3: 5% NaOCl + 40% S.persica.

The reason for group 1 showing low Ca/p ratio could be due to strong chelating action of EDTA when used alternatively with sodium hypochlorite, as study done by Marending M, Luder et al has shown removal of the organic phase from mineralized dentine by NaOCl enhances dentine permeability to EDTA23.

The reason for relatively higher Ca/p ratio of group 2 and group 3 compared to group 1 may be due to mild chelating action of S. persica as compared to EDTA. And hence eliminates smear layer without removing much of calcium and phosphate content.

When G2 and G3 were compared, mean Ca/p ratio was more for G2 than G3 although there was no statistically significant difference between them.

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G2 showing higher ca/p ratio compared to G3 might be because of difference in concentration of sodium hypochlorite used. Higher the concentration of hypochlorite more is the dissolution of organic phase of mineralized dentin which is in accordance with the study done by and zhang k et al24 Gu Ls et al[25].

Also, when coronal middle and apical third was compared apical third had maximum Ca/P ratio followed by middle third and then coronal third in all the 3 groups tested. The reason for apical third showing highest Ca/P ratio would be due to inability of irrigants to reach the apical third of root canal.

E. faecalis was used in the present study because it is believed that it is one of the intra-canal bacteria which are most resistant to elimination by disinfecting agents[26,27] .Antimicrobial property of Salvadora persica extracts is attributed to the different phytochemical constituents I.e, flavonoids, sterols, saponins, tannins & basic alkaloids[9].

Antimicrobial property of NaOCl is due to its Chlorine content (a strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulphydryl group) of essential bacterial enzymes2.

NaOCl and Salvadora individually has antimicrobial activity against E. faecalis as proven by siqueria et al2 and nawal A.k Al –sabawi et al[12].

Present study was done to assess antimicrobial activity of combined formulation of sodium hypochlorite and Salvadora persica.

Canals were flared with protaper rotary system F3 which helped in the better penetration of irrigating solutions into the canals to achieve maximum antimicrobial activity. To make it clinically relevant in our study we placed the experimental irrigant for 5min and 10 min in the infected canal .

Direct contact test was used in this study as it is simple to execute with good reproducibility that simulates contact between the bacteria and the irrigation solution inside the root canal[28].

Results of this study demonstrated that there was difference in mean value between the groups tested at 5 min interval i.e, 5% NaOCl + 40% S.persica was most effective followed 3% NaoCl and 3% NaOCl + 40% S.persica . But on statistically analysis no significant difference was found in antimicrobial efficacy between all the 3 groups at 5min.

At 10 min interval 5 % NaOCl + 40% S. persica showed highest mean followed by 3% NaOCl and 3% NaOCl + 40% S.persica . Also on statistical analysis statistically significant difference was found between 5% NaOCl + 40% S.persica and 3% NaOCl , 3% NaOCl + 40% S.persica. In both the time interval tested 5% NaOCl + 40% S.persica was found to be most effective antimicrobial agent against E faecalis. This might be on the account of higher concentration of sodium hypochlorite that was used .Results of this study is in accordance with the study done by Berber et al. who reported 5% NaOCl to be most effective antimicrobial agent against E. faecalis 29.

Also, the results showed improved antimicrobial activity of experimental irrigants at 10 min interval and 5% NaOCl+ 40 % S. persica to be most effective which might be due to increased contact time of the experimental irrigant with contaminated root canal.

Results of our study is in accordance with Gulashi et al who reported antimicrobial activity of NaOCl increases with increase in the time of contact 30 .

Our study concludes that combined formulation of 5% NaOCl + 40 % S.Persica is most effective root canal irrigant among the groups tested . 3% NaOCl + 40% S.persica is as effective as 3% NaOCl l& 17% EDTA in terms of antimicrobial, chelating agent, pulp dissolution property, while being less erosive on root dentin. Thus, both the experimental formulation (3% and 5% NaOCl + 40% S.persica) can be considered as a better alternative to 17% EDTA and 3% NaOCl

CONCLUSION

The ultimate goal is to look for a single irrigant that can combine the benefits of the traditional combination of sodium hypochlorite and EDTA with less adverse effect. While the results of our study seem favorable in terms of smear layer removal, pulp dissolution, antibacterial action, further long-term in-situ and in vivo studies are before it can be recommended as irrigant in routine clinical practice.

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Nil

CONFLICT OF INTEREST

There are no conflicts of interest

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