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Original Article

Evaluation of Antibacterial Effect Against E. Feacalis and Smear Layer Removal Ability of Turmeric Extract Solution as a Root Canal Irrigant for Primary Anterior Teeth: An In - Vitro Study

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Abstract

Objectives: The aim of this study was to evaluate and compare the antibacterial effect and smear layer removal ability of 12.5% turmeric extract solution, 2% chlorhexidine when used as root canal irrigants.

Materials and Methods: For antibacterial effect, seventy extracted primary anterior teeth were divided into five groups; Group (I): n= 20 roots that were irrigated with 2% chlorhexidine solution, Group (II): n=20 roots that were irrigated with sterile saline, Group (III): n=20 roots that were irrigated with 12.5% turmeric solution, Group (IV): positive control group (n=5 roots), Group (V): negative control group (n=5 roots). Sterile paper points were used to absorb irrigating fluid and transferred to a test tube to measure the bacterial count. For smear layer removal ability, 15 roots were divided into three groups (5 roots each). After instrumentation, the roots were irrigated with (12.5% turmeric extract, 2% CHX and sterile saline). The smear layer removal ability was evaluated by a scanning electron microscope.

Results: CHX group has a higher bacterial efficacy against E. faecalis than saline and turmeric irrigation solutions. The saline group had the highest (mean ±SD) total remaining smear layer followed by the turmeric group while the CHX group had the lowest (mean±SD).

Conclusion: Both 12.5% turmeric and 2% CHX irrigation solution have an antibacterial effects against E. faecalis and ability to smear layer removal. In infected canals, 2%CHX is the preferred irrigation choice, it has more effective antibacterial activity than 12.5% turmeric solution. Turmeric extract can be used as a safe natural alternative to CHX.

Keywords: Antibacterial properties, Irrigation, Primary teeth, Smear layer, Turmeric.

Introduction

Preservation of primary teeth until physiological exfoliation is a necessary factor that contributes to aesthetics, mastication and phonation. As well it prevents deleterious habits in children and helps the eruption of succeeding permanent teeth into an ideal position.[1,2,3]

Endodontic treatment aims to preserve the tooth in the dental arch in a healthy condition. This is highly important in children, as the early loss of primary teeth can compromise the development of the stomatognathic system and the installation of the permanent dentition as well as it can lead to emotional, psychological and behavioral problems. [3,4]

The success of endodontic treatment in primary teeth strongly relies on achieving an adequate level of disinfection within their root canals. Mechanical instrumentation alone is not sufficient to attain such disinfection, due to the persistence of significant number of pathogenic microorganisms within dentin debris and necrotic pulp-tissue

remnants inside the dentin tubules, canal ramification, and resorption craters. [5]

The use of chemical agents during instrumentation to completely clean all aspect of the root canal system is essential for successful endodontic treatment. Irrigation is complementary to instrumentation in facilitating the removal of pulp tissue and/ or microorganisms. [6]

Irrigation currently represents the best method in primary teeth pulpectomy for the lubrication and flushing away of loose necrotic and contaminated materials during instrumentation. [7]

In clinical practice, different intracanal irrigants have been proposed for primary teeth, as chlorohexidine gluconate (CHX), sodium hypochlorite (NaOCl), Ethylenediaminetetraacetic Acid (EDTA), (a mixture of tetracycline isomer, an acid and a detergent) MTAD, hydrogen peroxide (H_2O_2), and others.[8]

Considering the ineffective results, potential side effects and safety concerns of synthetic drugs, the herbal alternatives may prove to be advantageous for endodontic usage. [9]

Herbal and natural products have been used in dental and medical practice for thousands of years and have become even more popular nowadays because of their high antimicrobial activity, biocompatibility, antioxidant and anti- inflammatory proprieties. [10]

Turmeric has been used for thousands of years as a dye, a flavoring and a medicinal herb. In India, it has been used traditionally as a therapy for stomach and liver disease, as well as topically to heal sores. Ancient Indian medicine has described turmeric as an herb with the ability to provide glow and luster to the skin as well as vitality to the entire body. Since turmeric has antimicrobial, antioxidant and other useful properties.[11]

It is also useful in dentistry in different ways for relief dental problems such as pain and swelling , gingivitis and periodontitis, pit and fissure sealant, dental- plaque detection system .[11]

Few studies showed the efficacy of turmeric extract as a root canal irrigant solution in primary teeth. Therefore, the present study aimed to highlight the use of this material in the form of root canal irrigant solution, assess its antibacterial effect, smear layer removal ability and compare it with chlorhexidine.

Materials and Methods

Sample size calculation:

A power analysis was designed to have adequate power to apply a two- sided statistical test of the research hypothesis (null hypothesis) that there is no difference between the antibacterial efficacy of turmeric extract and chlorhexidine solutions as root canal irrigants in primary teeth against enterococcus faecalis. According to the results of **Chamele and Bhat** [12] assuming an alpha (α) level of 0.05 (5%), a Beta (β) level of 0.10 (10%) i.e. power =90%, and an effect size (f) of (0.47); the predicted sample size (n) was a total of (70) specimens i.e. (20) in each of the three experimental groups and (5) in each of the two control groups. Sample size calculation was performed using G*Power version 3.1.9.2

According to the results of **Balto et al.**[13] that there is no difference in smear layer removal capabilities of turmeric extract and chlorhexidine solutions as root canal irrigants in primary teeth and assuming an alpha (α) level of 0.05 (5%), a Beta (β) level of 0.10 (10%) i.e. power=90%, and an effect size (f) of (1.07); the predicted sample size (n) was a total of (15) specimens i.e. (5) for each group. Sample size calculation was performed using G*Power version 3.1.9.2 [14]

Assessment of the Antibacterial effect:

1. Selection of the teeth:

Seventy extracted primary anterior teeth were collected from the outpatient clinic of Pediatric Dentistry Department, Faculty of Dentistry, Ain Shams University and some private clinics. The teeth were selected according to the following criteria: [15] Minimum apical resorption (presence of at least 2/3 of root length), absence of root cracks and fractures, absence of internal, external root resorption as assessed by x-ray, absence of root caries. All teeth were extracted for reasons unrelated to the study such as extensive caries, avulsion and for orthodontic reasons. The teeth were discarded by the patients without writing consent. Teeth were stored in distilled water at room temperature until the experimental procedure (not more than three months).[16]

2. Specimens grouping:

Specimens were grouped into five groups as follows: [16] **group (I):** consisted of 20 roots that were contaminated with E. faecalis and irrigated with 2% chlorhexidine solution (Kempetro for chemical industries, Egypt), **group (II):** consisted of 20 roots that were contaminated with E. faecalis and irrigated with sterile saline, **group (III):** consisted of 20 roots that were contaminated with E. faecalis and irrigated with 12.5% turmeric solution, **positive control group (IV):** consisted of 5 roots that were contaminated only, **negative control group (V):** consisted of 5 roots that were not contaminated nor irrigated.[17]

3. Preparation of aqueous solution of turmeric:

The extract was prepared at Nawah Company, Almokattam Cairo, Egypt. The Curcuma longa rhizomes were washed with distilled water and dried, then, they were cut into irregular large pieces and dried in an oven by tray drying process at a temperature of 45±50 ºC for a period of about 9-10 days till they were completely moisture-free. The irregular large-sized pieces were ground into powder by using a ceramic mortar and an electrical blender to produce a fine powder.[18]

Two hundred and fifty grams of the powdered Curcuma longa rhizomes were placed into a 1 L conical flask then macerated with 1000 ml of absolute Ethanol.[19] The conical flask was then wrapped with aluminum foil and left overnight at room temperature. The resultant extract filtered through Whitman's filter paper (Whitman Ltd., England).[18]

For separating the ethanol from the extract, the flask was placed in a water path of ELABORATE 4000eco rotary evaporator (Heidolph Instruments GmbH& Co. KG, Germany).[19]

The resultant extract was let dry to provide 112.5 gms of yield extract. The Dilution was done by adding 1 part of it to 1 part of sterile distilled water. The resultant solution was used for the irrigation, it contained 12.5% turmeric extract.[19]

4. Preparation of specimens:

A rotary double-sided diamond disc (NTI diamond disc, Axis Denta 1, USA) mounted on a high-speed contra-angle with water coolant was used to de-coronate the teeth below the cementoenamel junction.[20]

Access cavity was prepared and K-file# 15(Mani, Japan) was placed in the root canal of each tooth until its tip appeared at the apical foramen to ensure patency of the canal, The length of the file was measured (root length) and the working length was then calculated and recorded by subtracting 1 mm from the anatomical root length of each tooth. [21]

The root canals were then instrumented using hand files (K-type) and enlarged to size #40 to standardize the diameter of the root canals. Irrigation of the canals with normal saline was performed after the use of each file to prevent blockage of the canal.[22] After canal preparation, the apical foramina of all the specimens were sealed from the external surface with α-cyanoacrylate adhesive (Amir Alpha co.) to prevent bacterial microleakage. [20,21]

5. Sterilization of the specimens:

All specimens were packed in sterilization pouches and autoclaved at 121 ºC and 15 PCI pressure for 15 minutes in Andromeda vacuum xp autoclave. [23]

6. Biofilm development:

The microbiological culturing was carried out in the Department of microbiology Faculty of Medicine, Ain Shams University, in which a clinical isolate of E. faecalis from the Microbiology laboratory (Central laboratories, Ministry of Health, Egypt) was used for biofilm formation.[24]

The prepared roots of the experimental groups and the positive control group were immersed in a 24-hour pure culture suspension of E. faecalis grown on Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroite, MI, USA) and adjusted to No. 1 MacFarland turbidity standard; all the roots were incubated at 37 ºC (HERAEUS B20 incubator) in sealed vials for 4 weeks.[24]

7. Irrigation of the specimens:

After contamination, samples were divided into 5 groups: 3 experimental groups, one positive control group and one negative control group. Each root was irrigated with 5 ml of the assigned group irrigant using (24G) sterile plastic syringes for 5 minutes.[25]

8. Bacterial sampling and bacterial count:

Sterile paper points were used to absorb the irrigation fluid in the canals. After 10-fold of serial dilution, aliquots of 0.1 ml were spread plated onto BHI agar plates and incubated at 37ºC for 48 hours.[24] Visible colonies of E. faecalis were counted on every plate and the number of colonies /plates was multiplied by the corresponding dilution factor and by 10 to determine the total colony-forming units (CFU) per ml of sample.[26]

Smear Layer Removal ability:

1. Specimens preparation and irrigation:

Fifteen extracted primary anterior teeth were selected for the study; the teeth were decoronated, instrumented and rinsed with 5ml distilled water. Then, after cleaning, shaping and drying of the root canal with an absorbent paper point, roots were randomly divided into three groups each group =**5**. The samples were dried with sterile paper points and were ready to receive the (final flush) irrigant.

The irrigations were delivered via a sterile 27 Gauge plastic syringe placed passively inside the canal 2mm from the working length. The irrigant was applied as follows: [27]

- **Group 1:** 5ml of 12.5% of turmeric solution was applied for 2min.
- **Group 2:** 5ml of 2% CHX was applied for 2 min.
- **Group 3:** 5ml of normal saline was applied for 2min.

2. Scanning Electron Microscopy (SEM):

The roots were split longitudinally into two halves through the two longitudinal grooves which were prepared both lingual and buccal surfaces by using a diamond disc without penetrating the canal. The roots were then split into two halves with a hammer and chisel. One half of each root was selected in which the entire canal was observed.[28]

The sections were mounted on metallic stubs, gold-sputtered, and examined under a scanning electron microscope (SEM) (JSM-5400LV, JEOL, TOKYO, Japan). All specimens were numbered, and the images were performed without knowledge of the group tested. Then, the most representative area of each third of each root was selected and magnified under different magnifications.

3. Evaluation of smear layer:

The evaluation of the smear layer by (SEM) was done in the regional centre for mycology and biotechnology at Al Azhar University**.** To evaluate the degree of smear layer removal ability, the results scored using ***modified Hülsmann et al*** [29] scoring system as follows:

Score 1= no smear layer, orifices of dentinal tubules are open.

Score 2= small amount of smear layer, most of the dentinal tubules are open.

Score 3= homogenous smear layer covering the root canal wall, a few of the dentinal tubules are open.

Score 4= the entire root canal wall is almost covered with a homogenous smear layer with very few open dentinal tubules.

Score 5= heavy, non-homogenous smear layer covering the complete root canal wall.

Statistical analysis:

Numerical data were presented by mean and standard deviation (SD) values and were explored for normality by checking the data distribution, calculating the mean and median values and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed non-parametric distribution and extreme positive skewness. Log transformation of the data was carried out to correct for the skewness. Leven's test showed a violation of variance homogeneity assumption so robust one-way ANOVA followed by Games Howell post hoc test was used for the analysis. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with IBM SPSS Statistics Version 26 for Windows.

Results

I-Bacterial count

The antibacterial efficacy of 12.5% turmeric extract solution used as intracanal irrigant in extracted primary teeth infected with E. faecalis was assessed and compared to 2% CHX digluconate, normal saline, negative and positive control groups. Results are presented as follows:

Intergroup comparison:

Mean and standard deviation (SD) values of log bacterial count (CFU/ml) for different groups were presented in table (2).

There was a significant difference between samples of different irrigation materials ($p<0.001$). The highest bacterial count was found in the positive control samples (5.31±0.07), followed by saline (4.58±0.25), turmeric solution (3.33±0.05), then Chlorhexidine samples (2.09±0.25), while no bacterial growth was found in the negative control samples. Pairwise comparisons showed values of samples irrigated with different materials to be significantly different from each other ($p<0.001$).

II-Smear layer removal ability

Intergroup comparison:

Mean and standard deviation (SD) values of smear layer removal score for different groups were presented in table (4).

There was a significant difference between samples of different irrigation materials ($p=0.004$). The highest score was found in saline samples (4.80±0.45) figure (3), followed by Turmeric (3.00±0.71) figure (1), while the lowest value was found in chlorhexidine samples (2.00±0.71) figure (2). Pairwise comparisons showed a value of saline samples to be significantly higher than that of chlorhexidine samples ($p<0.001$).

Discussion

In children, the main objective of root canal treatment is to remove completely the infected tissue and seal the canal(s) with a biocompatible material. In addition, completing the root canal procedure in a shorter time and at the same time providing a good quality treatment has always been the choice of interest for most practitioners.[30]

NaOCl, CHX, EDTA and H_2O_2 have been the most widely used root canal irrigating solutions. The main advantages of these chemical irrigants are their ability to dissolve necrotic tissues and their effective antibacterial properties against microorganisms. However, they have several undesirable characteristics such as tissue toxicity, risk of emphysema, allergic potential and disagreeable smell and taste.[31,32]

Turmeric pharmacological active components like flavonoids, phenolics and aromatics have antibacterial, antifungal, anti-inflammatory and anti-tumour properties. It is also available in Egypt and at a low cost. Therefore, it was chosen in this study as an irrigation material for primary teeth pulpectomy compared to CHX to assess their antibacterial effect against E. feacalis and smear layer removal.[33]

The 2% CHX concentration was used which is considered a high concentration in comparison to the 0.12% and 0.2% available in the market to increase the antimicrobial effectiveness. This was in agreement with **Gomes et al (2001)**[34] who stated that at higher concentrations CHX has a bactericidal effect while at low concentrations it has only a bacteriostatic effect. **Sassone et al (2003)**[35] also reported that a low concentration of CHX (0.12%) did not eliminate E.faecalis in any time interval.

In the current study, for antibacterial properties, the root canals of extracted primary teeth were infected with E.faecalis. Several studies have shown that E.faecalis is the predominant and most frequently isolated bacteria from necrotic pulps of primary teeth and its presence can contribute to the development of chronic infections such as periodontitis which can lead to early extraction of primary teeth.[36]

The microbial suspension used in the present study were adjusted to match the turbidity of No.1 MacFarland scale, to standardize the microbial suspensions used through the testing procedures as reported by **Sukawat et al. (2002)**[37] and **Sassone et al. (2008)**[38].

The presence of E.faecalis in root canals was detected by the culture method. This is considered to be useful as a primary investigation method to identify and quantify predominant species or make a correlation of some bacteria to certain clinical findings. This goes in agreement with **Cogulo et al. (2007)**[39] who used the same method.

This study was carried out in vitro as the isolation of E.faecalis bacteria from the root bacterial ecology is a very sophisticated process that may lead to unreliable data and results.

The five minutes duration of exposure to irrigating solutions was selected as it was found to

Table (1): Descriptive statistics for bacterial count (CFU/ml) of different groups

Measurement	Groups	Mean	SD	Median	Min.	Max.
Bacterial count (10^3)	**Turmeric**	2.52	1.12	2.00	1.40	5.00
	Chlorhexidine	0.13	0.09	0.10	0.00	0.30
	Saline	44.00	27.16	35.00	20.00	100.00
	Positive control	209.00	33.15	210.00	150.00	250.00
	Negative control	0.00	0.00	0.00	0.00	0.00
Log bacterial count	**Turmeric**	3.33	0.05	3.30	3.26	3.40
	Chlorhexidine	2.09	0.25	2.00	1.74	2.48
	Saline	4.58	0.25	4.54	4.30	5.00
	Positive control	5.31	0.07	5.32	5.18	5.40
	Negative control	0.00	0.00	0.00	0.00	0.00

Table (2): Mean ± standard deviation (SD) of bacterial count (CFU/ml) for different groups

Log bacterial count (mean±SD)					p-value
Turmeric	**Chlorhexidine**	**Saline**	**Positive control**	**Negative control**	
3.33 ± 0.05^{C}	2.09 ± 0.25^{D}	4.58 ± 0.25^{B}	5.31 ± 0.07^{A}	0.00 ± 0.00^{E}	**<0.001***

Means with different superscript letters are statistically significantly different*; significant ($p \leq 0.05$)
One-way ANOVA followed by Games Howell post hoc test.

Table (2): Descriptive statistics for smear layer removal score of different groups

Groups	Mean	SD	Median	Min.	Max.
Turmeric	3.00	0.71	3.00	2.00	4.00
Chlorhexidine	2.00	0.71	2.00	1.00	3.00
Saline	4.80	0.45	5.00	4.00	5.00

Table (4): Mean ± standard deviation (SD) of smear layer removal score for different groups

Smear layer removal score (mean±SD)			p-value
Turmeric	**Chlorhexidine**	**Saline**	
3.00 ± 0.71^{AB}	2.00 ± 0.71^{B}	4.80 ± 0.45^{A}	**0.004***

Means with different superscript letters are statistically significantly different*; significant ($p \leq 0.05$) One-way ANOVA followed by Games Howell post hoc test.

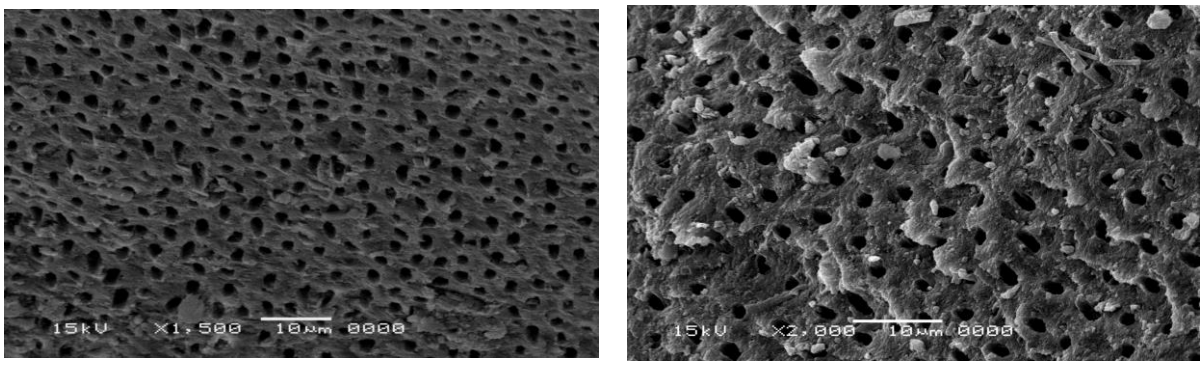

Fig. 1: SEM image showing smear layer removal by 12.5% turmeric solution

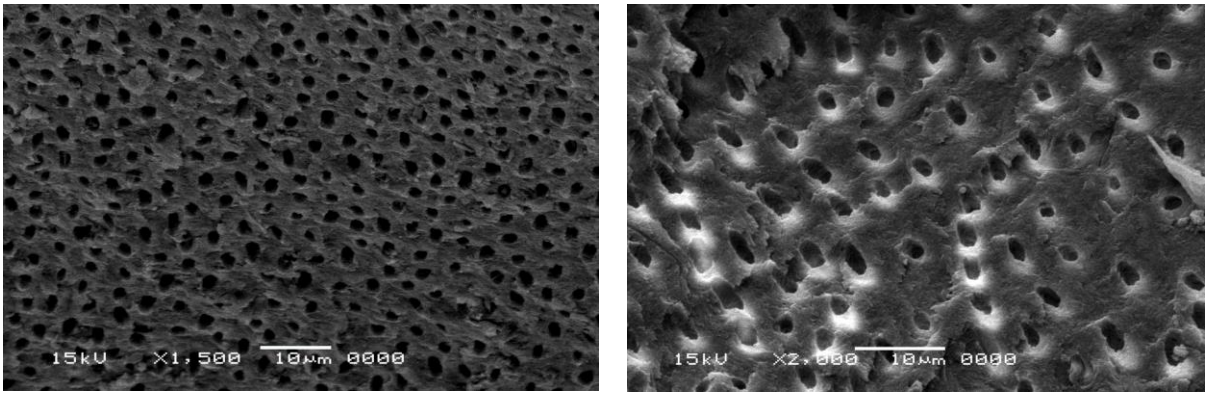

Fig. 2: SEM image showing smear layer removal by 2% chlorhexidine

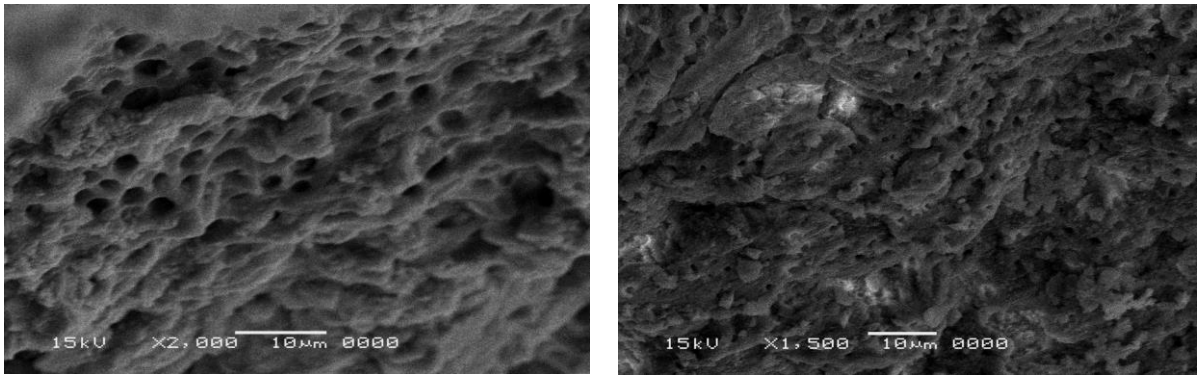

Fig. 3: SEM image showing smear layer removal by saline

be the optimum time for the irrigation material to assessment of the antibacterial effect on the basis of study by **Shabahang (2003)**.[21]

According to the results of the present study, both CHX and turmeric extract solutions showed antibacterial effects when compared with saline. There was a significantly higher antibacterial effect

of CHX irrigation than that of turmeric extract irrigation. This was in agreement with **Prabhakar et al. (2013)**[40] in which antimicrobial efficacy of 2% CHX was 95% compared with 54% with the turmeric extract group.

It is also worth mentioning that, the present study used roots of primary anterior teeth. Operating on roots of primary molars may show different results due to the presence of lateral canals, anastomoses and apical ramifications, in which other factors may contribute to the effectiveness of the antibacterial irrigation such as accessibility and substantivity.[41]

For smear layer removal ability, instrumentation techniques produce a smear layer and plugs of organic and inorganic particles of calcified tissues. The smear layer contains additional organic elements such as pulp tissue debris, odontoblastic processes, microorganisms and blood cells in the dentinal tubules. A smear layer can create a space between the inner wall of the root canal and the obturating materials, thus preventing the complete locking and adherence of the root canal filling materials into the dentinal tubules.[28]

Irrigation was done with 2% CHX, 12.5% turmeric solution and sterile saline. Standardization of these irrigant volumes and irrigation time was considered to attain similar conditions for all the specimens.[42] Irrigation time was 2 minutes each 2% CHX, 12.5% turmeric solution and saline solutions, because this seemed clinically practical.[43]

Smear layer removal was investigated by scanning electron microscope (SEM), where different types of microscopes can be used for evaluation of canal cleanliness, but the SEM is still the most common method for obtaining information about dentin surfaces. The main advantage of SEM is that it allows evaluation of the morphologic details of the surfaces of the prepared canal walls along their entire length.[44]

The results of the part concerning the remaining smear layer showed that the highest total remaining smear layer score mean value was recorded with saline. The results showed that the least total remaining smear layer score value was recorded with CHX then turmeric solution.

Irrigation with Saline could not remove the smear layer effectively. The dentin wall was still covered with a heavy smear layer, lacking any open dentinal tubules. This came in accordance with the finding of **Serafino et al.** [45] that indicated the final rinsing with Saline cannot achive efficient removal ability of the smear layer.

In contrast to present results, one study showed least smear layer removal with 2% CHX and saline groups in comparison to 10% EDTA and 5.25% NaOCl. This could be as a result of the irrigation protocol they used. During instrumentation, canals were irrigated with saline and final irrigation was done with 10 ml of 2% chlorhexidine gluconate.[46]

However, SEM evaluation has a limitation; assessment of only limited areas of the canal wall. Some attempts have been made to consider this potential limitation in as far as for each third the canal wall was completely screened under SEM and for each third of the canal always the area showing the greatest amounts of debris and smear layer was photographed and further analyzed. Another limitation of SEM evaluation is the bi-dimensional analysis of debris and smear layer. Thus, this method did not allow the measurement of the thickness of both residues.[27]

Conclusion

Both 12.5% turmeric and 2% CHX irrigation solution have an antibacterial effects against E. faecalis and smear layer removal ability. In infected canals, 2% CHX is the preferred irrigation choice, it has more effective antibacterial activity than 12.5% turmeric solution. Turmeric extract can be used as a safe natural alternative to CHX.

Conflict of interest:
The authors declare no conflict of interest.

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References

1. **Thakkar, T.K., Nai, K.S., Ghule, K. (2019):** Advances in Endodontics in Pediatric Dentistry. Review Article. EC Dent Sci.; 5(18):1320-1330.
2. **Thakur, B., Hospital, S.H.N., Pawar, A.M., Kfir, A., Neelakantan, P. (2017):** Extrusion of Debris from Primary Molar Root Canals following Instrumentation. J Contemp Dent Pract.; 18(11):1040-1044.
3. **Johnson, M.S., Britto, L.R., Guelmann, M. (2006):** Impact of a biological barrier in pulpectomies of primary molars. Pediatr Dent.; 28(6):506-510.
4. **Siqueira, J.F. and Rôças, I.N. (2008):** Clinical implications and microbiology of bacterial persistence after treatment procedures. J. Endod.; 34(11): 1291–1301.
5. **Kargül, B., Tanboga, I., and Altinok, B. (2010):** Conventional endodontic treatment of primary molars using metronidazole as an intra-canal medicament: a pilot study. Eur. Arch. Paediatr. Dent.; 11(4): 196– 200.
6. **Shen, Y., Stojicic, S., Qian, W., Olsen, I., and Haapasalo, M. (2010):** The synergistic antimicrobial effect by mechanical agitation and two chlorhexidine preparations on biofilm bacteria. J. Endod.; 36(1): 100–104.
7. **Hegde, V. and Kesaria, D.P. (2013):** Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E. Faecalis and C. Albicans–An in vitro study. Endodontology; 25(2): 38–45.
8. **Kaur, R., Singh, R., Sethi, K., Garg, S., and Miglani, S. (2014):** Review Article Irrigating Solutions in Pediatric Dentistry: Literature Review and Update. J Adv Med Dent Scie.; 2(2): 104–115.
9. **Pujar, M. and Makandar, S.D. (2011):** Review Article Herbal usage in endodontics. J of contempory dent.; 1: 2.
10. **Lin, C.P., Li, U.M., and Guo, M.K. (2006):** Application of Ni-Ti rotary files for pulpectomy in primary molars. J Dent.; 1(1): 10.
11. **Chamele, J. and Bhat, C. (2014):** Efficacy of turmeric extract as an intracanal medicament in deciduous teeth against Enterococcus faecalis: An in vitro study. Int. J. Curr. Microbiol. App. Sci.; 3(9):17-25.
12. **Chamele, J. and Bhat, C. (2014):** Efficacy of turmeric extract as an intracanal medicament in deciduous teeth against Enterococcus faecalis: An in vitro study. Int. J. Curr. Microbiol. App. Sci.; 3(9):17-25.
13. **Balto, H., Salama, F., Al-Mofareh, S., Al-Yahya, F. (2015):** Evaluation of different irrigating solutions on smear layer removal of primary root dentin. The journal of contemporary dental practice; 16(3):187-191.
14. **Faul, F., Erdfelder, E., Lang, A.G., Buchner, A (2007):** G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods.; 39(2):175-191.
15. **Cogulu, D., Atac, U.Z., Oncag, O., Aksoy, S., Eronat, C. (2017):** Detection of Enterococcus faecalis in necrotic teeth root canals by culture and polymerase chain reaction methods. European journal of dentistry; 1(04):216-221.
16. **Elnagar, M.H., Ghoname, N.A., Ghoneim, W.M. (2018):** Cleaning efficacy of rotary versus manual system for root canal preparation in primary teeth. Tanta Dental Journal; 15(1):14.
17. **Nara, A., Dhanu, P.C., and Latha Anandakrishna, D. (2010):** Comparative Evaluation of Antimicrobial Efficacy of MTAD, 3% NaOCI and Propolis Against E Faecalis. Int. J. Clin. Pediatr. Dent.; 3: 21-25.
18. **Vibha, H. and Dhaval, P.K. (2013):** Acomparative evaluation of antimicrobial effect of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E.faecalis and C.albicans-An in vitro study; 25(2):38-45.

19. Annegowda, H.V., Bhat, R., Min-Tze, L., Karim, A.A., Mansor, S.M. (2012): Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. Journal of food science and technology; 49(4):510-14.

20. Khademi, A., Usefian, E., Feizianfard, M. (2007): Tissue dissolving ability of several endodontic irrigants on bovine pulp tissue. Iranian endodontic journal; 2(2):65.

21. Shabahang, S., Torabinejad, M. (2003): Effect of MTAD on Enterococcus faecalis–contaminated root canals of extracted human teeth. Journal of Endodontics; 29(9):576-579.

22. Dametto, F.R., Ferraz, C.C.R., de Almeida Gomes, B.P.F., Zaia, A.A., Teixeira, F.B., and de Souza-Filho, F.J. (2005): In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against Enterococcus faecalis. Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology; 99(6): 768–772.

23. Ahangari, Z., Samiee, M., Yolmeh, M.A., Eslami, G. (2008): Antimicrobial activity of three root canal irrigants on enterococcus faecalis: an in vitro study. Iranian endodontic journal; 3(2):33.

24. Saber, S.E. and El-Hady, S.A. (2012): Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. European journal of dentistry; 6(01):043-50.

25. Nara, A., Dhanu, P.C., and Latha Anandakrishna, D. (2010): Comparative Evaluation of Antimicrobial Efficacy of MTAD, 3% NaOCI and Propolis Against E Faecalis. Int. J. Clin. Pediatr. Dent.; 3: 21-25.

26. El-Tayeb, M.M., Abu-Seida, A.M., El Ashry, S.H., El-Hady, S.A. (2019): Evaluation of antibacterial activity of propolis on regenerative potential of necrotic immature permanent teeth in dogs. BMC Oral Health; 19(1):1-2.

27. Urban, K., Donnermeyer, D., Schäfer, E., Bürklein, S. (2017): Canal cleanliness using different irrigation activation systems: a SEM evaluation. Clinical oral investigations; 21(9):2681-87.

28. Lotfi, M., Vosoughhosseini, S., Saghiri, M.A., Zand, V., Ranjkesh, B., Ghasemi, N. (2012): Effect of MTAD as a final rinse on removal of smear layer in ten-minute preparation time. Journal of endodontics; 38(10):1391-4.

29. Hülsmann, M., Rümmelin, C., Schäfers, F. (1997): Root canal cleanliness after preparation with different endodontic handpieces and hand instruments: a comparative SEM investigation. Journal of endodontics; 23(5):301-6.

30. Nerkar, R., Yadav, S., Metita, V., Joshi, P. (2015): Root canal preparation in in primary teeth: a review. J Adv Oral Res.; 6(2): 1-4.

31. Spencer, H.R., Ike, V., Brennan, P.A. (2007): The use of sodium hypochlorite in endodontics-potential complications and their management. Br. Dent. J.; 202(9): 555-9.

32. Becking, A.G. (1991): Complications in the use of sodium hypochlorite during endodontic treatment: report of theses. Oral surgery, oral Med. oral Pathol.; 71(3): 346–348.

33. Doddanna, S.J., Patel, S., Sundarrao, M.A., Veerabhadrappa, R.S. (2013): Antimicrobial activity of plant extracts on Candida albicans: An in vitro study. Indian Journal of Dental Research; 24(4):401.

34. Gomes, B., Ferraz, C.C.R., Berber, V.B., Teixeira, F.B., and Souza-Filho, F.J. (2001): In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int. Endod. J.; 34(6): 424– 428.

35. Sassone, L.M., Fidel, R., Fidel, S., Vieira, M., Hirata, R. (2003): The influence of organic load on the antimicrobial activity of different concentrations of NaOCl and chlorhexidine in vitro. International endodontic journal; 36(12):848-852.

36. Ozbek, S.M., Ozbek, A., Erdorgan, A.S. (2009): Analysis of Enterococcus faecalis in samples from Turkish patients with primary endodontic infections and failed endodontic treatment by real-time PCR SYBR green method. Journal of Applied Oral Science; 17:370-374.

37. Sukawat, C. and Srisuwan, T. (2002): A comparison of the antimicrobial efficacy of three calcium hydroxide formulations on human dentin infected with Enterococcus faecalis. Journal of endodontics; 28(2):102-4.

38. Sassone, L.M., Fidel, R.A., Faveri, M., Guerra, R., Figueiredo, L., Fidel, S.R., Feres, M. (2008): A microbiological profile of symptomatic teeth with primary endodontic infections. Journal of endodontics; 34(5):541-5.

39. Cogulu, D., Atac, U.Z., Oncag, O., Aksoy, S., Eronat, C. (2007): Detection of Enterococcus faecalis in necrotic teeth root canals by culture and polymerase chain reaction methods. European journal of dentistry; 1(04):216-21.

40. Prabhakar, A.R., Taur, S., Hadakar, S., Sugandhan, S (2013): Comparison of antibacterial efficacy of calcium hydroxide paste, 2% chlorhexidine gel and turmeric extract as an intracanal medicament and their effect on microhardness of root dentin: an in vitro study. International journal of clinical pediatric dentistry; 6(3):171.

41. Nair, P.N., Henry, S., Cano, V., Vera, J. (2005): Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology; 99(2):231-52.

42. Miranda, R.G., Santos, E.B., Souto, R.M., Gusman, H., Colombo, A.P. (2013): Ex vivo antimicrobial efficacy of the E ndo V ac® system plus photodynamic therapy associated with calcium hydroxide against intracanal E nterococcus faecalis. International endodontic journal; 46(6):499-505.

43. Saber, S.E. and Hashem, A.A. (2011): Efficacy of different final irrigation activation techniques on smear layer removal. Journal of endodontics; 37(9):1272-75.

44. Lotfi, M., Vosoughhosseini, S., Saghiri, M.A., Zand, V., Ranjkesh, B., Ghasemi, N. (2012): Effect of MTAD as a final rinse on removal of smear layer in ten-minute preparation time. Journal of endodontics; 38(10):1391-4.

45. Serafino, C., Gallina, G., Cumbo, E., Monticelli, F., Goracci, C., Ferrari, M. (2006): Ultrasound effects after post space preparation: a SEM study. J Endod.; 32: 549-552.

46. Hariharan, V.S., Nandlal, B., Srilatha, K.T. (2010): Efficacy of various root canal irrigants on removal of smear layer in the primary root canals after hand instrumentation: a scanning electron microscopy study. Journal of Indian Society of Pedodontics and Preventive Dentistry; 28(4):271.