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Original Research Article

Comparative evaluation of a novel herbal formulation with commercially available synthetic solution in cleaning used endodontic instruments: An in vitro study

Naveen Chhabra[1]*, **Desai Krupa**[2]

[1]*School of Dentistry, International Medical University, Kuala Lumpur, Malaysia*
[2]*Consultant Endodontist, Thane West, Mumbai, Maharashtra, India*

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ABSTRACT

Aim and Objectives: The study aimed to compare the cleaning effectiveness of a novel herbal solution with commercially available cleaning solution in eliminating biological debris from the surfaces of used endodontic instruments.

Materials and Methods: Thirty freshly extracted human single rooted premolars were decoronated to the standardized length. The root canals were prepared using rotary files till master apical size 35 (0.04 taper) and distilled water as irrigant. Used master apical files were assessed under scanning electron microscope (SEM) at 40x magnification at apical, middle and coronal third of working end independently for debris accumulation on the cutting surface of file and scored. All the specimens were randomly divided according to the group distribution as follows: Group 1: Synthetic cleaning solution, Group 2: Novel herbal cleaning solution. All files were immersed in the solution for five minutes according to the allocated groups and wiped using sterile gauze piece with one complete rotation covering all the flutes. Each used file was again examined under SEM at 40x at apical, middle and coronal third levels by an independent blinded evaluator and the residual debris were scored. The collected data were subjected to independent sample t test ($p<0.05$).

Results: Statistically significant difference was observed amongst the two groups at middle and apical third of endodontic files ($p < 0.05$), however, difference at coronal third level was insignificant ($p > 0.05$).

Conclusion: The herbal solution effectively removed the clogged debris from endodontic files as compared to commercial formulation.

1. Introduction

Thorough disinfection of clinical instruments during and after dental treatment is challenging due to abundance of diverse microbiota in the human oral cavity.[1] Endodontic instruments directly interact with the vital / necrotic tissues, dentinal remains and blood that may comprise of multiple infective microorganisms.

There is a greater risk of conducting pathogenic germs through endodontic instruments with compromised infection control.[2] The interchange of possibly residual debris over instruments from one patient to another or even one root canal to another in the same patient could be detrimental as it serves as an antigen, infecting agent, or a broad-spectrum irritant. The microorganisms isolated from the infected root canals may be detrimental to oral and systemic well-being of an individual.[3] The endodontic instruments carry the greatest hazard of cross contamination amongst individuals due to its extensive usage in general and specialty dental practice posing the greater risk of various bacterial, viral and the prion diseases.[4,5]

* *Corresponding author*.
E-mail address: drnaveenchhabra@yahoo.com (N. Chhabra).

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Complete elimination of the adhered dental residues over the used endodontic instruments is utmost critical. Occurrence of debris is often associated with the complex design of the files, that have fluted and twisted sections making mechanical and chemical cleaning considerably difficult.[6]

Various methods have been recommended for cleaning endodontic instruments that include mechanical cleaning using different kinds of brushes and sponges, chemical cleaning by immersion in varied sodium hypochlorite concentrations, enzymatic cleaners and detergents, hydrogen peroxide and combined use of chemo-mechanical aids or ultrasonics.[7]

Autoclaving is the gold standard sterilization practice in healthcare industry. However, adequate surface cleaning of instruments is an important prerequisite to achieve complete sterilization of the instruments. Various solutions are commercially available for presoaking and cleaning of instruments such as Asepti-Zyme^{TM} (Ecolab, India). It is a mixture of propylene glycol, alcohols (C12-14-secondary, ethoxylated), boric acid, subtilisin, lipase and triacylglycerol. The solution is low foaming, non-staining, and leaves no residue over instruments. The use of herbal products is on the rise in all avenues of healthcare, owing to the nominal side effects with no risk of developing antimicrobial resistance.[8]

Syzygium aromaticum or clove is commonly used as spice during various food preparations. It holds potential anti-inflammatory, antinociceptive and antipyretic effects.[9] Also, previous researches have described the antimicrobial effects of clove extracts against a wide variability of bacteria.[10–12]

Azadirachta indica (Neem) is possibly one of the most prevalent medicinal plants across Indian region. It's almost every part is traditionally consumed as household medicine against innumerable human disorders as it contains phytochemicals that hold exceptional antimicrobial and anti-inflammatory properties.[13]

Sapindus mukorossi (Sapindaceae) tree, also known as "ritha," "aritha" or "soapnut" is abundant across India. Its fruit's pericarp is majorly comprised of saponins (10–11.5%), sugars (10%), and mucilage. Saponins are known to lessen the surface tension and are frequently used in detergent preparations. Ritha solution is used to clean the oily secretion of skin, hair cleanser as well as a hair tonic. Apart from this, its seed extract has also been used for the treating dental caries[14] and its antibacterial, antifungal and anti- inflammatory actions are previously documented.[15] A recent laboratory study even established the capability of different extracts of Sapindus mukorossi in dissolving human dental pulp tissue.[16]

Citrus aurantifolia or lemon extract naturally contains roughly 5-6 % of citric acid as one of the components. Citric acid is a weak organic acid and chelating agent and well documented for removing dentinal debris and smear layer.[16,17] It is successfully tested in combination with the Sapindus mukorossi extract for smear layer removal in a former laboratory study.[14]

Despite the availability of numerous natural products for cleaning, the quest for an optimal herbal disinfectant is still going on.[18,19] No disinfecting agent is complete until it holds all desirable properties as antimicrobial, tissue dissolvent and smear and debris removing agent. Hence, the combination of different herbal agents with different capabilities appears to be an effective alternative.

Limited researchers have evaluated the cleaning efficacy of endodontic instruments using herbal preparations, thus, suggesting the need to search for a potent herbal preparation that stands equally effective or even better than commercially available synthetic cleaning aids in terms of its cleaning effectiveness and removal of biologic debris from used endodontic instruments. Hence, the present study was aimed to comparatively evaluate the cleaning effectiveness of commercially available cleaning aid and novel herbal solution comprising of extracts of clove, neem, reetha, and lemon.

The null hypothesis stated that novel herbal solution's ability to remove biological debris deposited on used endodontic instruments will be no better than commercially available synthetic cleaning aid.

2. Materials and Methods

The necessary approval was obtained prior to the commencement of the study from Institutional Ethics Committee (SVIEC/ON/DEN/SRP/18025). Thirty freshly extracted human single rooted intact premolars, extracted for orthodontic or periodontal reasons were collected from department of oral and maxillofacial surgery. All the teeth were disinfected by immersing the specimens in 0.1% thymol solution for one week and cleaned using ultrasonic scaler. All the teeth were decoronated to obtain the uniform length of 15 mm. After obtaining canal patency using size 10 K file (Mani Inc, Japan), the specimens were prepared with NiTi rotary endodontic instrument (DXL-pro, Dexell, Mumbai, India) and endodontic motor (TriAutoMini, J Morrita, Japan) in a sequential manner up to apical size 35 (0.04 taper). Distilled water was used as an irrigating solution between each instrument. The used instruments were kept aside for 24 hours to allow the accumulated biologic debris to get dried and settled. All the used files were assessed under scanning electron microscope at 40X magnification at three levels of working end of instrument; apical, middle, and coronal third and any presence of accumulated debris over the file surface was identified by an experienced independent blinded evaluator (Figure 1A-C, Figure 2A-C). The final scores were tabulated using modified evaluation format adapted from the scoring system suggested by Parente et al. (2010) as follows:[18] Score 1-

No debris; Score 2- 25% or more but less than 50% of file covered with debris; Score 3- 50% or more but less than 75% of file covered with debris; Score 4- 75% or more but less than 100% of file covered with debris; Score 5- 100% debris covering the file. Thereafter, all the specimens were randomly divided using flip coin method according to the group distribution as follows: Group1: Instruments cleaned using commercially available synthetic cleaning solution (Aseptic Zyme, Ecolab, India) Group2: Instruments cleaned using novel herbal cleaning solution.

*2.1. Preparation of novel **h**erbal solution*

The 100ml of herbal composition was prepared using clove oil (Nilgiris Clove oil, India), neem oil (KhadiPure, India), ritha extract (Navchetan Kendra, New Delhi) and citrus extract (Navchetan Kendra, New Delhi) in aqueous base. 16 mg of Sapindus extract and 20 mg of Citrus extract were diluted in 100 ml of distilled water. A mixture of clove oil and neem oil (20ml each) was taken and slowly mixed into the aqueous mixture. The proportioning of the ingredients was done in such a way that resulted in completely miscible solution without any precipitation. The formulation was prepared using emulsification method owing to the oil and aqueous component. The mixture was continuously stirred for 30 minutes using manual method (ceramic mortar and pestle) until a uniform mix of all the components was achieved. Thereafter, 100 ml of prepared herbal solution was pipetted and stored at room temperature.

All the used files were immersed in the solution for five minutes as per the allocated respective groups and wiped in 2 x 2 inch^2 size gauze with one complete rotation involving all the flutes. The immersion time of five minutes was standardized for either group as per the manufacture recommendations for manual cleaning for Aseptic Zyme. The presence of any remaining debris in manual cleaning were determined by examining each file under SEM at 40x at three levels: apical, middle, and coronal third (Figure 1D-F, Figure 2D-F). Image evaluations were performed by an experienced independent blinded evaluator and the presence of debris were scored again as discussed previously.

3. Results

The independent t test was used to comparatively assess the outcome for both the groups using SPSS software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). The mean difference scores for group 1=2.6, and group 2=3.67 at apical third differed statistically ($p< 0.05$). Similarly, the mean difference scores for group 1=3.07 and group 2=3.73 at middle third were also statistically different ($p< 0.05$). However, the coronal third scores for both groups 1= 2.67 and group 2=3.27 were statistically insignificant ($p> 0.05$). (Table 1)

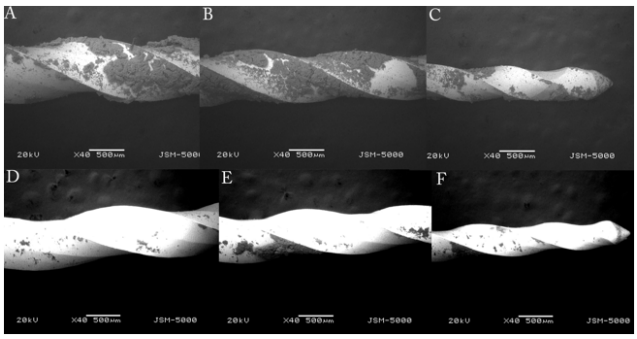

Figure 1: Representative SEM Images (40X) of used specimens (Group1) before immersing in the solution: (**A-C**): coronal, middle and apical respectively, Group 1 after immersion in the respective test solution (**D-F**): coronal, middle and apical respectively.

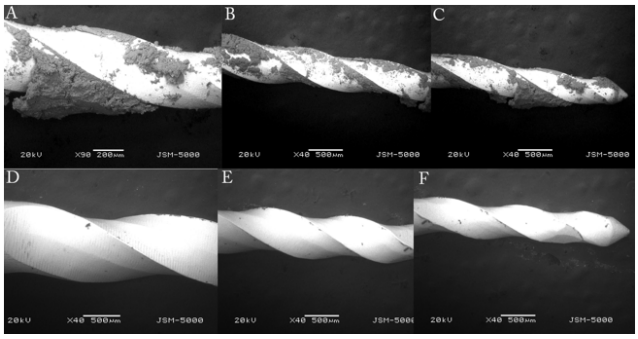

Figure 2: Representative SEM Images (40X) of used specimens (Group2) before immersing in the solution: (**A-C**): coronal, middle and apical respectively, Group 2 after immersion in the respective test solution (**D-F**): coronal, middle and apical respectively.

4. Discussion

The complex designs of endodontic files essentially demand a cleaning method that can proficiently serve the purpose in minimum possible time. The accumulated debris within the file flutes is difficult to clean owing to the concomitant presence of mineralized and organic contents. The novel herbal solution was prepared by the combination of different herbal extracts, anticipating the synergistic outcome. In this experiment the quantity of residual debris was significantly reduced in all the groups following a single immersion in the cleaning solution. However, the novel herbal solution considerably cleaned the debris in apical and middle third of the instrument in comparison to the existing commercial solution. Considerably greater volume of debris in the apical third of the file specimens may be attributed to the compact fluting in the apical section of the files. Also, the greater apical debris in the master file may be linked to the fact that only the apical end is active during the cutting action. Better results with novel herbal combination are attributed to the emulsification of the bio-surfactants like S. mukorrossi which is efficacious in dissolving the water-

Table 1: Independent Students t Test for comparison of the two groups

	Group	Total sample size	Mean Score	Std. Deviation	P Value
Before Solution Coronal	Group 1	15	2.73	0.799	0.078
	Group 2	15	3.27	0.799	
Before Solution Middle	Group 1	15	3.27	0.799	0.101
	Group 2	15	3.73	0.704	
Before Solution Apical	Group 1	15	3	1	0.055
	Group 2	15	3.67	0.816	
After Solution Coronal	Group 1	15	0.07	0.258	0.334
	Group 2	15	0	0	
After Solution Middle	Group 1	15	0.2	0.561	0.189
	Group 2	15	0	0	
After Solution Apical	Group 1	15	0.4	0.632	**0.028**
	Group 2	15	0	0	
Difference Coronal	Group 1	15	2.67	0.816	0.051
	Group 2	15	3.27	0.799	
Difference Middle	Group 1	15	3.07	0.961	**0.039**
	Group 2	15	3.73	0.704	
Difference Apical	Group 1	15	2.6	0.91	**0.002**
	Group 2	15	3.67	0.816	

insoluble substances.[20] Soapnut solution is regarded as very effective and mild at the same time. The natural surfactant action of Sapindus mukorossi resultant to the abundance of saponins, has been extensively used for cleaning the oily skin, hairs and metallic jewelries as well.[21]

The rebirth of the herbal age is happening around the globe. The natural products are considered safe for humans, in contradiction to the currently available synthetic formulations. In dentistry the use of natural extracts as anti-inflammatory, antimicrobial and antiplaque agents is well documented.[22] The way antibodies are indispensable component of human immune system, plants also secrete essential oils as self-protective mechanism. These essential oils have been extensively used in naturopathy to treat various disease since ages.

Lime extracts and lime essential oils are frequently used in perfumes, cleaning products, and aromatherapy. The traditional and pharmacological uses of Citrus aurantifolia plants are credited to the presence of several plant metabolites including flavonoids, coumarins, and terpenoids. Biologists have recently become increasingly interested in the useful biological actions of essential oils, especially their broad antimicrobial abilities against variety of pathogenic microbes.[23]

In the present study, citrus aurantifolia extract was used as it contains citric acid which has capacity to remove smear layer and additionally showing antimicrobial effect. Preliminary research was undertaken to evaluate the efficacy of the unique combination of Citrus aurantifolia as chelating agent and Sapindus mukorossi as a surfactant. The results of the study justified the synergistic efficacy of this combination in removing the debris and smear layer from the root canal walls.[14]

Previous research has evidenced the higher efficacy of clove oil against oral and systemic pathogens.[1] The clove oil has previously shown better results in comparison to clove extract against the foodborne pathogens.[24] Clove oil causes disruption of the cell membrane leading to leakage of cellular constituents and finally bacterial cell death. Traditionally clove oil is used as antiseptic and analgesic in dentistry. It is active against bacteria causing dental caries and effective against many other bacteria and virus. Documented literature provides information about biological activities like insecticidal, antifungal, anti-mutagenic, antioxidant and antiallergic as well.[1]

Elavarasu et al. in their study found neem oil to be effectively diminishing the progress of the plaque-causing microorganisms.[25] Bohora et al. also reinforced the significant antimicrobial effect of neem extract. Neem contains terpenoids like nimbin, alkaloids, tannins, and flavonoids.[26] These phytochemicals cause inactivation of proteins and cell function by binding to adhesins forming complex in bacterial cell wall.[27]

The results of our experiment justified the worth of prepared novel combination and its applicability to the dental disinfection as compared to commercially available synthetic solution. Currently enzymatic detergents are extensively suggested for cleaning of medical devices as they remove proteins, lipids and carbohydrates from the instrument plane. It contains propylene glycol, alcohol and subtilisin. It is a faster and easier procedure for cleaning dental instruments. Subtilisin is widely used in the detergent industry for the removal of protein-based stains like egg, milk, or blood.[28]

In the current study, for debris evaluation SEM was used at the magnification setting of 40x. Although, the currently available optical microscopes such as stereomicroscope

are sufficient to view debris present on the file at this magnification setting, the decision to use SEM was taken to eliminate the possibility of obscured visualization of debris from the flutes caused due to reflected light. Also, the SEM produces a three-dimensional visualization of the sample being examined. However, stringent standardization approach was followed in designing and evaluation, the study is prone to possible risks of operator related errors, as the study utilized manual method to remove debris using gauze piece which is prone to operator fatigue and difficult to standardize for each cleaning cycle. Hence, further studies utilizing standardized and repeatable approach using mechanized devices like ultrasonic bath may be designed. The present study did not assess the antimicrobial effect of the combination hence, further research is needed for the same.

5. Conclusion

Within the limitations of present research herbal solution was effectively able to remove debris from endodontic files. The method could effectively serve the purpose of chairside disinfection during intermittent usage of instruments and as a presoaking solution before autoclaving. Autoclaving remains the standard benchmark for sterilization process until its antimicrobial efficacy is proven in further research. Also, nano-formulations of the same along with detailed phytochemical analysis of the prepared solution may be deemed necessary to improve the quality and shelf life of the final solution.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Naveen Chhabra, Senior Lecturer https://orcid.org/0000-0002-0174-4875

Desai Krupa, Consultant Endodontist

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