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RESEARCH ARTICLE

Comparative Evaluation Of the Smear Layer Removal Efficacy Of Andrographis Paniculata, Indigenously Prepared Herbal Extract (Endopam) When Used As Root Canal Irrigants; An In-Vitro Study

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Abstract: Smear layer removal is an essential step in root canal therapy, as its presence can obstruct dentinal tubules and reduce the effectiveness of irrigants, medicaments, and sealers. Conventional agents such as EDTA and sodium hypochlorite are effective but present concerns related to cytotoxicity and adverse effects on dentin, prompting interest in herbal alternatives. Plant-based irrigants are valued for their natural origin, antimicrobial potential, chelating ability, and improved biocompatibility. Andrographis paniculata is noted for its antibacterial, anti-inflammatory, and antioxidant properties that may contribute to cleaner dentinal surfaces. EndoPam, an indigenously prepared herbal formulation, offers an eco-friendly and cost-effective approach with potential advantages in endodontic applications. The exploration of these herbal extracts reflects the broader movement toward safer, biocompatible, and sustainable irrigating solutions for effective smear layer management in modern endodontics.

INTRODUCTION

Root canal instrumentation produces a smear layer [1] which is composed of a superficial layer (1-2 µm) on the root canal walls and a deep layer (40 µm) packed into dentinal tubules [2] which blocks the dentinal tubule orifices hindering irrigants, intracanal medicaments, and sealer penetration into dentinal tubule hampering fluid-tight seal of root canal treatment [3].

To decrease the risk of microleakage, smear layer should be removed; otherwise, it can lead to endodontic failure[4]. In this aspect, irrigants play a key role in the removal of smear layer[2].

For smear layer removal, instrumentation must be supplemented with irrigation [2].

Various synthetic chemical substances are being used as endodontic irrigants for their effectiveness in removal of smear layer and disinfection but also have undesirable properties such as toxicity and allergic potential [4]. Therefore, there is an increase in the exploration and use of natural phytochemicals to be a part of dental treatment procedures due to ease in availability, cost-effectiveness and better biocompatibility [4,5].

Sodium hypochlorite (NaOCl) has remained as gold standard for root canal irrigation because of its antimicrobial potential and its ability to dissolve organic matter. Nevertheless, it is not only irritant to the periapical tissues but also possesses disadvantages such of instruments, burning of surrounding tissues, unpleasant taste, high toxicity, corrosive to instruments, inability to remove the smear layer, reduction in elastic modulus, and flexural strength of dentin.[6]

Ethylenediaminetetraacetic acid (EDTA) was introduced and patented by Munz in 1935. It functions by forming a calcium-chelate solution with the calcium ion of dentin; the dentin thereby becomes more friable and easier to instrument. Chelating agents are available in both liquid and paste form.[7]

Andrographis paniculata, an annual herbaceous plant, popularly known as 'king of bitters' belongs to the Acanthaceae family. [8] It is also infamously called as Bhui-neem, which means "neem of the ground" as it has a similar strong bitter taste as that of the large neem plant. It is commonly cultivated in southern Asia, in China, and in some parts of southeast Asia. Being a potent stimulator of the immune system, it is effective against several viruses and bacteria and also a powerful antimalarial and antidiarrheal. [9]

In the ancient Ayurveda, many herbs with potent therapeutic effects including antibacterial, antifungal, analgesic and anti-inflammatory properties have been used such as M. piperita,[10] E. globulus,[11] C. zeylanicum,[12] S. aromaticum [13].These have been widely used for several medicinal purposes, however their role in endodontics have not been evaluated till date. Hence, in this present study these were combined to prepare an indigenous product, to achieve a potential additive or synergistic effect, as an endodontic irrigant. [14]

Comparing the efficacy of these herbal agents is not reported. Hence, the purpose of the study is to compare the smear layer removal efficacy of A.paniculata, indigenously prepared herbal extract (Endopam) when used as root canal irrigants.

MATERIALS AND METHODS

- A total of 40 non-carious single rooted human permanent teeth extracted for periodontal or orthodontic purpose were collected for study. Teeth with carious lesion, severe attrition , fractures, cracks & developmental defects, multiple roots, calcified canals and open apices were excluded.

HERBAL IRRIGANT SOLUTIONS PREPARATION

For the preparation of Endopam, the fine powders of S.aromaticum(clove), C.zeylanicum (cinnamon), E.Globulus (eucalyptus / Tasmanian blue gum), M.piperita (peppermint) were collected. These powders were mixed with 50 ml each of absolute ethanol. The mixture was macerated for 1-2 min. to form an extract. Then, this extract was filtered through muslin cloth for coarse residue and then through Whatman filter paper no.41 for finer residue. Fine powder of A.paniculata were also collected and the same method was followed.The solutions were collected and kept in a dark bottle and stored at 40^{o}C until use.

GROUP 1: Normal saline
GROUP 2: 17% EDTA and 3% NaOCl regimen
GROUP 3: Andrographis paniculata ethanolic extract
GROUP 4: Endopam ethanolic extract

TOOTH PREPARATION

The teeth were cleaned using an ultrasonic scaler followed by sterilization with autoclave. The teeth were stored in distilled water until use.

The working length was measured radiographically to be 1mm short of the apical foramen. The Glide path was established using a size 15 k-file. All samples were randomly divided into 4 groups with each group having 10 samples (2 control group and 2 experimental group).

Root canals were prepared up to apical size 25, 0.06 taper using Protaper NiTi files at specific speed and torque by manufacturer`s instructions using the crown down preparation method. During instrumentation, canals were irrigated using 5ml of prepared solution corresponding to the respective group. In group 2, irrigation was done as follows ; 17% EDTA– Normal saline -3% NaOCL. In all the groups, study specimens were finally rinsed with normal saline and dried using sterile absorbent paper points. Samples were stored at humidity.

Then, teeth were decoronated at cementoenamel junction using diamond disc to obtain a standardized root length of 10mm. One longitudinal groove was placed on buccal and lingual surfaces on each root using diamond disc, avoiding penetration into the canal. The roots were then split into 2 halves using a chisel and mallet.

SCANNING ELECTRON MICROSCOPIC EVALUATION:

Specimens were mounted onto metallic stubs with carbon strip backing and viewed under Scanning Electron Microscope. Scanning Electron Microscope photomicrographs were taken at a magnification of x1500 to evaluate smear layer removal and the Rodig et al scoring system (Table 1) was used for evaluation.

Table 1; Rodig et al scoring system for smear layer removal evaluation

Score	Description
1	No smear layer , dentinal tubules open
2	Small amount of smear layer, some dentinal tubules open
3	Homogenous smear layer covering root canal surface wall, only a few dentinal tubules open
4	Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules
5	Heavy inhomogeneous smear layer covering the complete root canal wall

STATISTICAL ANALYSIS:

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0 Released 2013 Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis includes expression of CFUs, Smear Removal Scores & Debris Scores in terms of mean and standard deviation for each group.

Inferential Statistics:

One-way ANOVA Test followed by Tukey's Post hoc test / Kruskal Wallis Test followed by Dunn's post hoc Test [Based on Data Distribution] was used to compare the mean CFUs, Smear Removal Scores & Debris Scores between different groups.

The level of significance [P-Value] was set at $P<0.05$

And any other relevant test, if found appropriate during the time of data analysis were dealt accordingly.

RESULTS ;

The mean smear layer scores for the groups (Table 2) were:

1. **NaOCl +EDTA :** 1.90 ± 0.22 (Min: 1.63, Max: 2.24)
2. **Endopam:** 2.53 ± 0.23 (Min: 2.20, Max: 2.86)
3. **A. paniculata:** 3.93 ± 0.24 (Min: 3.57, Max: 4.44)
4. **Saline:** 4.71 ± 0.34 (Min: 4.21, Max: 5.36)

One-way ANOVA was used to compare mean scores among the 4 groups.

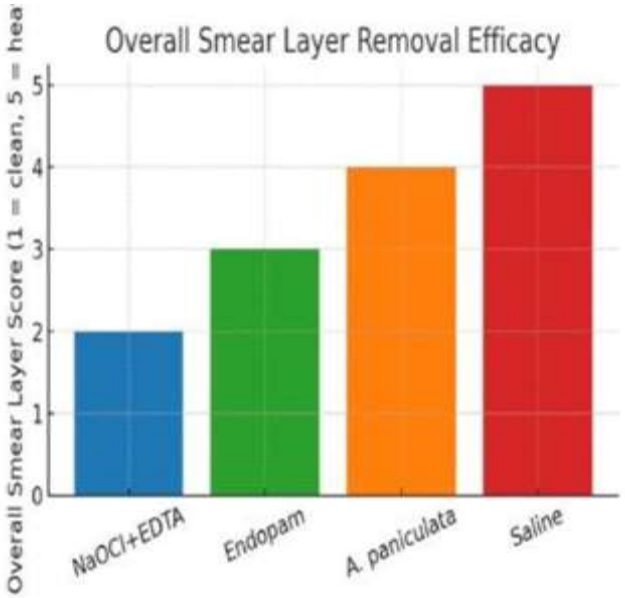
- **F = 237.31, p < 0.0001** → highly significant difference.

NaOCL + EDTA: Cleanest canals, most open tubules, best overall cleaning (Mean 1.90).
Endopam: Intermediate efficacy, several open tubules with some smear retention (Mean 2.53).
A. paniculata: Heavier smear layer than Endopam, fewer open tubules (Mean 3.93).
Saline: Thickest smear, almost no visible tubules, poorest cleaning of all groups (Mean 4.71).

The overall ranking of efficacy is:
NaOCL + EDTA > Endopam > A. paniculata > Saline

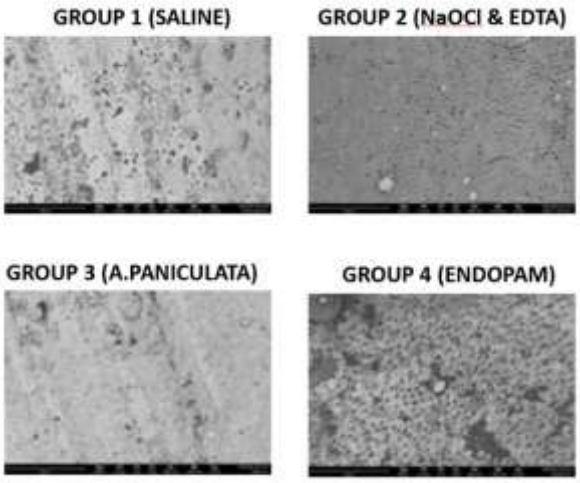
Table 2; Mean, standard deviation, and statistical analysis of smear layer removal by different irrigants

Group	Mean Score	Standard Deviation	Minimum	Maximum
NaOCl+EDTA	1.90	0.22	1.63	2.24
Endopam	2.53	0.23	2.20	2.86
A. paniculata	3.93	0.24	3.57	4.44
Saline	4.71	0.34	4.21	5.36



Graph representing overall smear layer removal efficacy by different irrigants

SEM



ANALYSIS photomicrographs of different irrigant groups under 1500x magnification

DISCUSSION ;

Indian traditional medicine was dependent on herbs and herbal products, and recently it has gained importance. The major advantages of herbal alternatives are easily available, inexpensive, less side-effects and lack of microbial resistance. [13]

The current study appears to be the first report comparing the endodontic applications of A. paniculata and Endopam.

The herbal agent A.paniculata exhibited antibacterial, antifungal, and antiviral effectiveness in previous reports.[16,17,18] It has proved antibacterial activity against Gram-positive(+) and Gram-negative(−) bacteria including avirulent Mycobacterium smegmatis and M. tuberculi. It also has antiinflammatory, immunostimulant and antipyretic effects,[19] which helps in the treatment of acute upper respiratory tract infections. It is known to have anticancer and immunomodulatory effects on human cells. It also shows significant antimicrobial and antioxidant properties, which was the basis of considering this extract as a prospective endodontic irrigant in our study. [8,19]

In the present study, four potent antimicrobial herbs were used as a combination (ENDOPAM) to be tested as a root

canal irrigant. S. aromaticum which is commonly known as clove, is found in the Maluku islands in east Indonesia. This plant is widely used in pharmaceutical, cosmetic, food and agricultural applications since it is rich in pharmacologically active compounds such as eugenol, eugenol acetate and gallic acid. [20]

Compounds such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, [21] β- pinene, γ-terpinene, α-terpinol, alloocimene and aromadendrene [22] give eucalyptus extracts their antibacterial activity. Recent studies have shown many potentially beneficial health effects of cinnamon such as anti-inflammatory properties, anti-microbial activity, blood glucose control, reducing cardiovascular disease, boosting cognitive function, and reducing risk of colonic cancer. [23] M. piperita extract has been reported to have antioxidant [24] and antimicrobial properties.[25]

Over the years, NaOCl and EDTA, as chelating agents, became the most often employed agents for disinfecting the root canal.

The clinical efficacy of sodium hypochlorite (NaOCL) for the irrigation of root canal space in endodontic therapy makes it the gold standard.[21] It has a unique property of dissolving necrotic as well as vital tissues.[22] However, there are some disadvantages such as toxicity, [23] disagreeable smell and taste,[24] and potential for allergic reactions.[25]

Clarkson concluded that irrigation with the solution of EDTA followed by NaOCl is most effective in the removal of the smear layer. However, it has certain disadvantages; it damages all living tissues except keratinized epithelium and also erodes the metal. [26]

The present study considered the 5 min as irrigation time following a study conducted by Divia et al. [27] A conventional 5 ml syringe was used for irrigation which was considered to be the most widely used technique according to Buldur and Kapdan.[28] and Hata et al.[29]

SEM, which can detect accurate surface characteristics, was used to assess the effectiveness of irrigants in the SL removal[30] by SEM Photomicrographs and was first reported by Eick et al.[31] Debris which was defined as dentin chips, pulp remnants, and particles loosely attached to the root canal wall,[32] can easily be observed at low magnification. Higher magnification is, however, required for the identification of dentinal tubules and observation of the remnants of the SL.

There are few limitations to be considered, such as need of fresh herbal extract preparation, limited samples were subjected to SEM examination. These results cannot be applied to multirooted teeth as they have anatomic complexity, which poses a challenge for root canal disinfection.

CONCLUSION ;

Within the limitations, it was found that endopam ethanolic extract showed comparable results similar to EDTA and NaOCL regimen. So, it can be stated that alternate use of Endopam ethanolic extract can serve as an alternative available natural extract for irrigation. The observations of herbal products appear promising. The results can be further justified by a larger sample size, and clinical trials before Endopam and A.Paniculata use can be recommended conclusively as an intracanal irrigating solution.

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