ABSTRACT

**Aim:** To assess the efficacy of various irrigants used with Self-Adjusting File (SAF) system on smear layer.

**Materials and Methods:** Forty extracted mandibular teeth were taken and decoronated. All samples were instrumented manually up to number 20 K file. SAF file was further used to prepare the canals. All the samples were randomly divided into four Groups: **Group I:** Neem Extract **Group II:** Amla Juice; **Group III:** 5.25% NaOCl+ 20% Citric Acid; **Group IV:** 5.25% NaOCl solution was used for 4 minutes. All roots were sectioned longitudinally in the buccolingual plane and viewed under electron microscope.

**Statistical Analysis Used:** The Kruskal-Wallis test was used for statistical evaluation and Mann-Whitney U test was used for multiple comparisons

**Results:** Comparison between same thirds of group showed statistically significant difference in coronal and middle parts. Group 3 showed the best results for smear layer removal. Group 4 showed least efficacy of smear layer removal.

**Conclusion:** Group 3 showed the best removal efficacy followed by group 1 and 2 with least being for group 4.
Keywords: Amla juice; citric acid; neem-leaf extract; self-adjusting file; sodium hypochlorite; smear layer.

1. INTRODUCTION

What we remove from the pulp space, is far more important than what we replace it with. Adequate debridement of the root canal plays an important role in the success of root canal treatment [1].

Biomechanical preparation of root canal produces "smear layer". The first researchers to describe the smear layer on the surface of instrumented root canals were Mc Comb & Smith. They suggested that the smear layer consisted not only of dentine as in the coronal smear layer, but also the remnants of odontoblastic processes, pulp tissue and bacteria. Lester & Boyde described the smear layer as ‘organic matter trapped within translocated inorganic dentine’. The debris removal is mandatory but the removal of the smear layer remains a controversial issue [2,3].

For optimal effectiveness, irrigants must be brought into direct contact with the entire root canal wall [4], and it was stated that enhancement of the flushing action is necessary to improve root canal cleanliness [5]. The organic tissue-dissolving activity of NaOCl is well known and increases with rising temperatures. However, the capacity to remove the smear layer from the instrumented root canal walls has been found to be insufficient. Many authors have concluded that the use of NaOCl during or after instrumentation produces superficially clean canal walls with the smear layer present [6]. Several chemicals have been investigated as irrigants to remove the smear layer. The most commonly used irrigation solutions are chelating agents and acids. The effectiveness of citric acid for removal of the smear layer was demonstrated in the 1970s [7-10]. The use of herbal alternatives as a root canal irrigant might prove to be advantageous considering the undesirable characteristics of NaOCl such as the cytotoxic and genotoxic effects on human peripheral lymphocytes. Also the extensive use of antibiotics can generate drug-resistant bacteria and it is necessary to develop new materials in order to overcome this problem.

Self-adjusting file (SAF) was introduced in 2010 and uses a hollow NiTi file, without a central metal core, through which a continuous flow of irrigation is provided.

Hence, the present study was undertaken to check the efficacy of various irrigants used with SAF system on smear layer using scanning electron microscope.

2. MATERIALS AND METHODS

2.1 Preparation of Neem Extract

Neem leaf powder was weighed (15 g), mixed with distilled water (150 ml), and boiled at 100°C to get 15 ml of neem leaf extract. The prepared solutions were filtered using Whatman filter paper, and the final irrigating solutions were obtained and stored in opaque bottles.

2.2 Specimen Preparation

Forty (n = 40) intact single-rooted human permanent teeth (n = 40) having a single canal and fully developed apices, indicated for extraction due to orthodontic/periodontal reasons, were selected for this study (Fig. 1). The teeth were then stored in distilled water until use. The coronal parts of the teeth were cut with a high-speed diamond bur to standardize the root lengths (Fig. 2, 3) and to provide direct access to the root canals. Number 15K files (Dentsply-Maillefer, Ballaigues, Switzerland) were introduced further into the root canals until their tips were visible at the apical foramen. The working length was determined as 1mm shorter than this length. The canals were instrumented manually up to number 20K file. 5 mL, 5.25% sodium hypochlorite (NaOCl) was used for irrigation between the instruments.

Fig. 1. Sample size
A SAF file system (ReDent-Nova, Israel) (Fig. 4) was used to prepare the root canals as described by Metzger et al.[9]. Irrigation was performed continuously during the instrumentation using a special irrigation apparatus (VATEA Irrigation Device, ReDent-Nova, Israel). This apparatus has two separate irrigant reservoirs connected to a hollow SAF file. Continuous irrigation was applied at a flow rate of 5 mL/min. The SAF file instrumentation with irrigation was performed for a total of 4 minutes in each root canal. All the samples were then randomly divided into four groups with each group having ten samples.

**Group I**: Neem Extract was used for 4 minutes (at a flow rate of 5 mL/min, 20mL in total).

**Group II**: Amla Juice was used for 4 minutes (at a flow rate of 5 mL/min, 20mL in total).

**Group III**: 5.25% NaOCl was used for 3 minutes (at a flow rate of 5 mL/min, 15mL in total) and then 5mL 20% citric acid was used for 1 minute.

**Group IV**: 5.25% NaOCl solution was used for 4 minutes (at a flow rate of 5 mL/min, 20mL in total).

Finally, all roots were irrigated with 5mL distilled water, then dried with sterile paper points, and left to dry at a room temperature for 24 hours. All roots were grooved longitudinally on the external surface with a diamond disc in the buccolingual plane, avoiding penetration of the root canals. The roots were separated into two halves with a chisel. The specimens were fixed on metal holders and coated with gold and viewed with scanning electron microscope (JEOL JSM-6510LV).

The most accessible areas in each third were selected and photomicrographed. The smear layer was evaluated from images at 1000x magnification based on the scale of Hulsmann et al. [10]: score 1, no smear layer and all dentinal tubules were open; score 2, a small amount of smear layer and some dentinal tubules were open; score 3, homogeneous smear layer covering the root canal wall and only a few dentinal tubules were open; score 4, complete root canal wall covered by a homogeneous smear layer and no open dentinal tubules; and score 5, heavy homogeneous smear layer covering the complete root canal. Scores 1 and 2 represent “clean canal wall.” Scores 3, 4, and 5 represent “smear layer present”.

The Kruskal-Wallis test was used for statistical evaluation and Mann-Whitney U test was used for multiple comparisons.
2.3 Statistical Analysis

The data was tabulated and statistically analyzed using SPSS Statistics V22.0 (IBM, USA). The score values were subjected to Kruskal-Wallis and Mann-Whitney test. The significance was set at $p < 0.05$.

3. RESULTS

Scanning electron microscopic images for all groups are shown (Figs. 5, 6, 7, 8 for Group 1, 2, 3, 4 respectively).

![Fig. 5. SEM micrograph of Group 1](image1)

![Fig. 6. SEM micrograph of Group 2](image2)

![Fig. 7. SEM micrograph of Group 3](image3)

![Fig. 8. SEM micrograph of Group 4](image4)
The SAF, operated with Group 3 (citric acid+NaOCl) and Group 1 (neem group), resulted in clean canals and most of the specimens revealed scores 1 and 2. Group 4 exhibited heavy smear layer covering the root canal walls.

3.1 Kruskal-Wallis Test

Mann-Whitney Test was performed for intergroup comparison which showed Group 3 had statistically significant difference compared to Group 2 & 4. Also Group 1 had statistically significant difference compared to Group 4. Group 1 & 2 had difference in only means (Table 1) but no statistically significant values for intergroup comparison.

4. DISCUSSION

A predominant trend in modern dentistry has been to search for biocompatible agents, especially those to be used in direct contact with tissues. In this context, phytotherapy has evolved as a science, and there has been growing interest in evaluating plant extracts with a potential therapeutic application in dentistry [11]. Sodium Hypochlorite (NaOCl) has an extensive history in medicine and dentistry and continues to be popular even today. NaOCl was moderately effective against bacteria but less effective against endotoxins in root canal infection.

The Indian neem/margosa tree is rich in its antioxidant properties and is an effective alternate to NaOCl in root canal irrigation [12].

Neem, Triphala and Amla all showed the potential to remove the smear layer. Although there is general agreement regarding the necessity of removing the smear layer, the optimal irrigation solution and removal technique remain under debate. The present findings revealed the effectiveness of the SAF system with four different irrigation solutions, suggesting that this methodology may be a useful alternative to conventional methods. Further studies are required to determine the most effective parameters.

Sebatni et al. [13] did a study to evaluate the smear layer removal efficacy of various herbal extracts and found that neem extract showed the best results due to the presence of acid metabolites, flavanoids.

Amla (Emblicaofficinalis) contains chemical ingredient Vitamin C, carotene, nicotinic acid, riboflavin, and tannins. A major constituent of Amla is also gallic acid. It is reported to possess hepatoprotective and antioxidant activity. The superior efficacy of smear layer removal with Amla could be a result of its low pH [14].

Numerous investigations revealed that extended exposure to acids results in excessive demineralization. Therefore, 4 min. of 20% citric acid application was used in the current study. Although we used a lower concentration of citric acid (20%), the smear layer was successfully removed in the majority of our specimens. This success can be attributed to the continuous irrigation and vibration action of the SAF system.

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Metzger et al. claimed that the SAF file has a scrubbing action on the canals, which clearly results in a very clean surface even in the unreachable parts of the canal by activation of the irrigant in the apical third of the canal. In a recent study of MeloRibeiro et al. [15], oval SAF was used with continuous NaOCl irrigation on oval-shaped root canals. These researchers reported that the percentage of remaining debris and uninstrumented canal perimeter was significantly lower in the SAF group than in the rotary group.

Table 1. Mean of smear scores related to the thirds of teeth in Groups 1, 2, 3 and 4 (intergroup comparisons of thirds)

|          | CORONAL     | MIDDLE     | APICAL     |
|----------|-------------|------------|------------|
| Group 1  | 1.80±0.02   | 2.00±0.21  | 2.10±0.14  |
| Group 2  | 2.20±0.32   | 2.30±0.09  | 2.60±0.1   |
| Group 3  | 1.40±0.42   | 1.80±0.01  | 2.10±0.7   |
| Group 4  | 2.40±0.26   | 2.80±0.57  | 3.00±0.90  |
Table 2. Comparison of the Same Thirds between Groups (Intergroup Comparisons of Thirds)

| Test Statistics<sup>a,b</sup> | Coronal | Middle | Apical |
|-------------------------------|---------|--------|--------|
| Chi-Square                    | 14.625  | 9.078  | 4.608  |
| Df                            | 3       | 3      | 3      |
| Asymp. Sig.                   | 0.002<sup>*</sup> | 0.049<sup>*</sup> | 0.107  |

<sup>a</sup> Kruskal Wallis Test  
<sup>b</sup> Grouping Variable: Group

Table 3. Comparison of Group 1 vs Group 2

| Test Statistics<sup>a</sup> | Coronal | Middle | Apical |
|-------------------------------|---------|--------|--------|
| Mann-Whitney U                | 33.000  | 41.000 | 31.000 |
| Wilcoxon W                    | 88.000  | 96.000 | 86.000 |
| Z                             | -1.592  | -1.773 | -1.574 |
| Asymp. Sig. (2-tailed)        | .111    | .439   | .116   |
| Exact Sig. [2*(1-tailed Sig.)]| .218<sup>b</sup> | .529<sup>b</sup> | .165<sup>b</sup> |

<sup>a</sup> Grouping Variable: Group  
<sup>b</sup> Not corrected for ties

Table 4. Comparison of Group 1 vs Group 4

| Test Statistics<sup>a</sup> | Coronal | Middle | Apical |
|-------------------------------|---------|--------|--------|
| Mann-Whitney U                | 26.000  | 24.000 | 25.000 |
| Wilcoxon W                    | 81.000  | 79.000 | 80.000 |
| Z                             | -2.072  | -2.134 | -2.088 |
| Asymp. Sig. (2-tailed)        | .038<sup>*</sup> | .033<sup>*</sup> | .037<sup>*</sup> |
| Exact Sig. [2*(1-tailed Sig.)]| .075<sup>b</sup> | .052<sup>b</sup> | .063<sup>b</sup> |

<sup>a</sup> Grouping Variable: Group  
<sup>b</sup> Not corrected for ties

<sup>*</sup> Highly significant difference  
<sup>•</sup> Significant difference
Table 5. Comparison of Group 2 vs Group 4

| Test Statistics     | Coronal | Middle | Apical |
|---------------------|---------|--------|--------|
| Mann-Whitney U      | 40.000  | 33.000 | 41.500 |
| Wilcoxon W          | 95.000  | 88.000 | 96.500 |
| Z                   | -.951   | -1.397 | -.680  |
| Asymp. Sig. (2-tailed) | .342   | .163   | .496   |
| Exact Sig. [2*(1-tailed Sig.)] | .481<sup>b</sup> | .218<sup>b</sup> | .529<sup>b</sup> |

<sup>a</sup> Grouping Variable: Group  
<sup>b</sup> Not corrected for ties

Table 6. Comparison of Group 3 vs Group 1

| Test Statistics     | Coronal | Middle | Apical |
|---------------------|---------|--------|--------|
| Mann-Whitney U      | 33.000  | 42.000 | 47.500 |
| Wilcoxon W          | 88.000  | 97.000 | 102.500|
| Z                   | -1.450  | -.659  | -.209  |
| Asymp. Sig. (2-tailed) | .147   | .510   | .835   |
| Exact Sig. [2*(1-tailed Sig.)] | .218<sup>b</sup> | .579<sup>b</sup> | .853<sup>b</sup> |

<sup>a</sup> Grouping Variable: Group  
<sup>b</sup> Not corrected for ties
Table 7. Comparison of Group 3 vs Group 4

| Test Statistics | Group   | Coronal | Middle | Apical |
|-----------------|---------|---------|--------|--------|
| Mann-Whitney U  |         | 12.000  | 20.000 | 27.000 |
| Wilcoxon W      |         | 67.000  | 75.000 | 82.000 |
| Z               |         | -3.130  | -2.387 | -1.817 |
| Asymp. Sig. (2-tailed) |      | .002*** | .017** | .069  |
| Exact Sig. [2*(1-tailed Sig.)] |     | .003^b  | .023^b | .089^b |

\* Grouping Variable: Group
\^ Not corrected for ties

Table 8. Comparison of Group 3 vs Group 2

| Test Statistics | Group   | Coronal | Middle | Apical |
|-----------------|---------|---------|--------|--------|
| Mann-Whitney U  |         | 16.000  | 34.000 | 34.000 |
| Wilcoxon W      |         | 71.000  | 89.000 | 89.000 |
| Z               |         | -2.952  | -1.309 | -1.270 |
| Asymp. Sig. (2-tailed) |      | .003**  | .190   | .204  |
| Exact Sig. [2*(1-tailed Sig.)] |     | .009^b  | .247^b | .247^b |

\* Grouping Variable: Group
\^ Not corrected for ties

* Highly significant difference
** Significant difference
The VATEA peristaltic pump used in the SAF system delivers a continuous flow of irrigant, which enters the canal through the hollow file [16].

According to the manufacturer, the motion of the file agitates the irrigant to such an extent that it effectively reaches the apical part of the canal with sonic activation. The irrigant can be delivered into the tube at a rate ranging from 1-10 mL per minute, with the typical recommended setting of 4 mL per minute. We thought that continuous replacement of irrigant could also explain the excellent cleaning efficiency observed in this study.

5. CONCLUSION

Present study concluded that comparison between same thirds of group showed statistically significant difference in coronal and middle parts. Group 3 (hypo+citric) showed the best results for smear layer removal. Group 4 (hypo) showed least efficacy of smear layer removal. Within the limitations of my study, we can say that herbal irrigants can provide an effective alternative to the chemical irrigants available in the market. The present findings revealed the effectiveness of the SAF system with 4 different irrigation solutions, suggesting that this methodology may be a useful alternative to conventional methods. Further studies are required to determine the most effective parameters.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline patients consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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