In vitro assessment of natural extract as irrigant on root dentin microhardness

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ABSTRACT

To determine the effect of a natural extract of stevia rebaudiana of 2.5% concentration on the microhardness of root canal dentine when used as an irrigating solution. This was an in vitro comparative study where hundred and twenty intact single-rooted teeth were selected and decoronated with a diamond bur to get an apical-coronal length of 10 mm and were randomly separated into three groups as per the irrigant used; Group 1 (control): Irrigation with Normal saline, Group 2: Irrigation with Smear Clear solution, Group 3: Irrigation with 2.5% Stevia extract solution. They were prepared using Pro Taper Universal Rotary Files with intermittent irrigation with the respective irrigating solution. The teeth were then embedded in acrylic resin and subjected to Vicker’s Hardness test, and the data obtained were analyzed using one way ANOVA. When Group 2 (51.80±9.80 VHN and 52.66±5.28 VHN) was compared with Group 3 (44.98±4.34 VHN and VHN) the values showed a statistically significant difference (p < 0.05). When Group 3 (44.98±4.34 VHN and VHN) was compared with Group 1 (65.37±8.80 VHN and 67.74±9.56 VHN), the values did show a statistically significant difference (p > 0.05). Within the limitations of this study, teeth irrigated with normal saline showed the least reduction in microhardness followed by irrigation with Stevia leaf extract solution and Smear Clear.

INTRODUCTION

Cleaning of all contents of the root canal system before and during shaping in endodontic treatment is one of the critical steps in endodontic (Hargreaves et al., 2016). To achieve ample success in debridement of canal necessitates the instruments to remove substances physically. Which is achieved by irrigating solutions which acts synergistically? Ideally, these irrigating solutions should possess numerous properties which are beneficial to the outcomes of endodontic treatment. Still, as it is known to all till date, no such irrigating answer is present as ideal.

As far as cleaning is concerned, the chances of success in the endodontic therapy exponentially increase, as and when more debris and smear layer are removed due to which detachment of microbiota and their associated toxins from root canals, helps in
providing 3D obturation and reduces the chances of survival of any potential of bacteria and their subsequent reproduction (Kandaswamy and Venkateshbabu, 2010).

One of the most extensively utilized chemical irritants is sodium hypochlorite (NaOCl) with a wide range of concentration from 0.5% - 5.25%. Regardless of its exceptional antimicrobial act and capability of liquefying inorganic materials, it is still lack in the removal of smear layer which makes it dependent on chelating agents that are expedient against organic matter (Silva et al., 2008). However, this leads to exposure of collagen fibres and a resultant decrease of dentin micro hardness (De-Deus et al., 2006).

Stevia rebaudiana is a natural sweetener to lower blood sugar (Goyal et al., 2010). Ethanolic and methanolic extracts of stevia leaves have been recognized to be operative in contrast to the Gram-negative and Gram-positive organisms (Ajaganananavar et al., 2014). Its odourless, colourless and sweet to taste properties make it superior to hypochlorite. The fact that it has under no circumstances been used as an irritant, which makes it ideal for assessment which employ the use of Stevia in this particular role in endodontic cure. Therefore with the intention of evaluating the effect of a natural extract of stevia leaves on microhardness of root dentin, this study was designed.

MATERIALS AND METHODS

120 non-carious with single root only premolar teeth undergone extraction for periodontal or orthodontic reasons were selected based on relative dimensions, the similarity in morphology and absence of any cracks or caries defects especially within the root portions and was verified radiographically. To maintain the standardization crowns were sectioned with high-speed bur under water cooling and apico-coral length of 10 mm was achieved. The mean mesiodistal and buccolingual dimensions were obtained after measuring the diameters of the coronal planes. After that, roots presenting a difference of 20% from the mean were discarded, leaving a total of 80 root samples.

Specimen preparation

Root canal length of all specimens was determination was using #10 K file till it was just visible at the apex. After the establishment of working length by reducing by 0.5 mm from that length. A prepared solution of 2.5% concentration of Stevia was then used as an irritant. At this stage, the teeth were randomly alienated into three groups (n=40) as per their exposure to different irrigants (Table 1);

Group 1 (control): Normal Saline as an irrigant, Group 2: Smear Clear TM (Kerr) solution as Irrigant, Group 3: 2.5% Stevia extract solution (Herboveda, India) as an irrigant, Biomechanical preparation was achieved by rotary ProTaper Universal Files (Dentsply, Maillefer) up to size F3 with sporadic irrigation between successive files, with 2 ml of the respective irrigant. Through a 27 gauge needle, each of the irrigants was reaching about 2/3rds the working length with a final rinse using normal saline to wash off any leftovers before testing.

Prepared specimens were embedded in self-cure acrylic resin, up to 8 mm, exposing 2 mm of their remaining length. With the help of pumice, slurry exposed flat surface was achieved. All the specimens were then subjected to Vicker’s Hardness testing at 500 and 1000 microns from the pulp-dentinal junction on the polished surfaces (Mitutoyo America Corporation, Load = 100-gram force, Dwell time= 15 sec). Three readings were documented at 500 and 1000 microns respectively, and an average was taken as the final reading at both distances (Mali et al., 2020a).

Data analysis

Data were collected, and statistical analysis was done using one way ANOVA at p < 0.05, which was taken to be statistically significant. All analyses were performed using SPSS (Statistical Package for Social Sciences) software version 20 (Mali et al., 2020b)

RESULTS AND DISCUSSION

Lower Vickers microhardness values were obtained at 500 microns compared with 1000 microns from the pulp space in all groups, without significant difference (p> 0.05) (Table 2).

Between the groups, the control group showed the highest microhardness at 500 and 1000 microns, namely, 65.37 8.80 VHN and 67.74 9.56 VHN, respectively. Group 2 and 3 showed a comparable reduction in microhardness with Group 1 showing slightly better results. Group 2 showed the least microhardness. When Group 2 was compared with Group 3, the values showed a statistically significant difference (p < 0.05). When Group 3 was compared with Group 1, the values did show a statistically significant difference (p > 0.05).

After scrutiny of the outcomes of this study, it may establish that root canal irrigation with chemical or natural composition irrigant except normal saline reduced the dentin microhardness. Lower Vickers microhardness values were acquired at 500 microns compared with 1000 microns from the pulp space.
Table 1: Specimen distribution as per irrigating solutions used

| Irrigating Solution                  | Group (n = 40) |
|--------------------------------------|---------------|
| Normal Saline (Control)              | 1             |
| SmearClear                           |               |
| 2.5% Stevia extract solution         | 3             |

Table 2: Microhardness values in vhn

| Group                  | 500 microns | 1000 microns |
|------------------------|-------------|--------------|
| Normal saline          | 65.37±8.80  | 67.74±9.56   |
| SmearClear             | 51.80±9.80  | 52.66±5.28   |
| Stevia leaf extract    | 44.98±4.34  | 45.89±5.26   |

These outcomes bolster past discoveries demonstrating that dentin microhardness is area related, and its value is diminished as the spaces are nearer to the pulp (Ferrer-Luque et al., 2013). The more significant number of extensively opened dentinal tubules, deprived of peritubular dentin near the pulp, presented little conflict to the indenter. As per testified by Pashley et al. microhardness of dentin and tubular density shows inverse relation. The intrinsic hardness profile of the dentin structure is rested upon the degree of mineralization and the amount of hydroxyapatite in the intertubular substance (Pashley et al., 1985).

Determination of microhardness can be considered to deliver an incidental indication of mineral loss or gain in dental hard tissues. The conducted study relied on a Vickers microhardness test, as it has shown suitability and practicality previously in the evaluation of surface variations of dental tissues subjected to chemical agents (Clark-Holke et al., 2003). The substantial modification in the microhardness of dentin, after irrigation, specifies that there are direct effects of these chemical solutions on dentin structure and its components.

In the study conducted by Ulusoy and Görgül (2013) Smear Clear was used to assess its consequence on root dentin microhardness, smear layer amputation and erosion among other endodontic irrigants and found that it was effective in eliminating the smear layer in the middle and cervical sections and also did not cause a substantial decrease in the root dentin microhardness. Aranda-Garcia AJ et al. reported a reduction in the microhardness after using Smear Clear with an explanation as it contains 17% EDTA, cetrimide and surfactant. These constituents can eliminate the smear layer of the root canals; however, its chelating ability is lower than the 17% EDTA (Aranda-Garcia et al., 2013). It probably explains a lesser reduction in the microhardness as compared to the 2.5% NaOCl + EDTA 17% group as shown in previous studies.

Stevia has gained its popularity in ancient times for various purposes as a bio-sweetener and to lower blood sugar without calories and sweeter than table sugar[8]. Because of its properties such as colourless, odourless and sweeter than sugar proves it suitable for use in endodontic procedures. Usha C et al. assessed the anticariogenicity of microwave-assisted 0.5% extract of Stevia rebaudiana leaves reported that 0.5% concentration can be acknowledged as a mouthwash for patients with moderate to high caries risk (Usha et al., 2017).

Escobar et al. testified that S. mutans total growth and biofilm formation showed a marked decrease with reduced concentrations of Stevia along with reduced biofilm and acid production (Escobar et al., 2020). When it comes to microhardness, Maryam Kishta et al. reported a loss of microhardness of bovine dentin with the use of 0.5% and 5% stevia extract (Kishta-Derani et al., 2012). As per evidence provided by the above studies, the anticarcinogenic potential of Stevia is verified when used as a mouth rinse. Still, there have been no studies concerning its use as an endodontic irrigant. An irrigant like Stevia which has anticariogenic potential, indicating a decrease in the biofilm formation and acid creation alongside an attractive taste that doesn't necessarily diminish the root dentin microhardness could amend the field of endodontic.

CONCLUSION

With some of the limitations of this study, observations suggest that canal irrigation with chemical or natural solutions leads to structural changes as evidenced by the reduction of dentine microhard-
ness. The extent of softening and demineralization may influence the physical and chemical behaviour of this heterogenic arrangement. It could be concluded Stevia leaf extract gives the impression of having a lesser effect on the root dentin microhardness when compared to smear clear which shows after more investigations and evidence stevia leaf extract be considered as an endodontic irrigant.

ACKNOWLEDGEMENT

Nothing to report.

Conflict of interest

Nil.

Source of funding

Self.

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