Treatment of Dentinal Hypersensitivity Using Propolis Varnish: A Scanning Electron Microscope Study

Abstract

**Background:** Dentin hypersensitivity (DH) affects all age groups in a population and is perceived as pain to any stimuli. From time immemorial, researchers have sought herbal or natural solutions to treat hypersensitivity. Propolis is one such natural, nontoxic resinous substance produced by honey bees, which is useful in various applications in dentistry and effective in treating dentinal hypersensitivity. **Aim:** The aim of this *in vitro* study is to assess the effect of propolis varnish on occlusion of dentinal tubules thus aiding in the treatment of dentinal hypersensitivity. The objective is to evaluate the effectiveness of the proposed treatment using scanning electron microscopy (SEM) imaging. **Materials and Methods:** Twenty naturally extracted teeth were collected and stored until *in vitro* assessment. Discs obtained from each tooth were divided into two groups of 20 each – I (test) and II (control), with each tooth acting as its own control. Propolis varnish was applied only to the acid-etched surface of the exposed dentin of discs in the test group, whereas all the discs were subjected to SEM imaging. **Results:** Statistical analysis showed a significant reduction in open tubules (*P* < 0.001) from 160 ± 6.97 before treatment to 61.20 ± 9.10 after propolis varnish application in the test group. **Conclusion:** This study showed the promise of propolis varnish as a natural treatment modality for DH.

Keywords: Dentinal hypersensitivity, herbal desensitizing agent, propolis varnish

Introduction

Dentin hypersensitivity (DH) is one of the most frequently encountered complaints of patients in dental practice. While 4%–74% of the population is affected by DH, it is more prevalent in the age group of 20–40 years.[1] Dentine exposure may result from the following factors: (i) loss of enamel or cementum and (ii) loss of gingival tissue by various physical and/or chemical processes. Causative factors that aggravate DH include acidogenic diets, destructive habits, poor tooth brushing techniques, erosion, abrasion, attrition, bruxism, bleaching, medication, aging, genetic conditions, gingival recession, and periodontal disease or procedures.[2,3] While it is capable of affecting the oral care of any individual, DH lacks a clinically proven efficient treatment modality which could be termed as the gold standard.

DH results in acute pain, arising from the exposed dentin in response to stimuli, which are typically thermal, evaporative, tactile, osmotic, or chemical; which is not explained by any other form of dental defect or pathology.[4,5] Some synonyms used for DH are dentin sensitivity, dentinal hypersensitivity, cervical hypersensitivity, root hypersensitivity, or cemental hypersensitivity.[4]

Several hypotheses were proposed to explain the mechanism of DH. Hydrodynamic theory, proposed by Gysi[6] and explained further by Brannstrom[7] remains the most widely accepted one. Based on this theory, DH reduces with the decrease in fluid flowing within dentinal tubules.[8] Pashley (1986) suggested that DH might be reduced physiologically by the formation of intratubular crystals from dentinal fluids and saliva minerals.[8,9] Several treatment strategies, namely, desensitization of nerve endings, masking of dentin tubules, occlusion of dentin tubules, and iontophoresis, are prevalent in clinical practices. A few examples are potassium nitrate, calcium silicate, stannous fluoride, strontium chloride, varnishes, composites, lasers, and periodontal soft-tissue grafting.

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Alternatively, homeopathic medication including *Plantago major* and propolis are also seen as effective modalities.[10] These agents are available in the form of toothpaste or gels, which are used for treating DH.

Use of natural products has found a wide range of applications in dental practices in the recent past. Propolis is one such product that refers to “a substance in defense of hive.” It is derived from Greek words *pro* that refers to “in front of” or “at the entrance to” and *polis* that refers to “community” or “city.”[11] Egyptians were the first to use propolis for medicinal purpose, which was later followed by the Greeks and the Romans.[12]

Varnish is a preparation that differs in the polymeric matrix, pharmaceutical additive, and therapeutic agents, which are generally a combination of fluoride and chlorhexidine. A polymer is used to provide consistency to the varnish, which is similar to that of a thin film. Among the most widely used are ethyl cellulose and a mixture of copolymer and vinyl acetate-acrylate copolymers.[13]

Propolis is a natural, nontoxic, resinous substance, which is yellow-brown. This is collected by honey bees from sprouts, buds, sap flows, exudates of trees, and other accessible parts of the plants. It is further modified in the bee hives by addition of salivary secretions of bees to form a sticky product known as beeswax or bee glue.[11,14] This glue provides an immune system to the bee hives by acting as a sealant, whose chemical composition depends on the origin of the plants.[15] Chemically, propolis consists of about 50-60% resins, 30-40% wax, 5-10% essential oils, and the remaining is pollen. It is made up of 300 organic compounds in addition to microelements such as aluminum and calcium.[14] Chemical compounds belong to the categories of phenolic acids and their esters, flavonoids, terpenes, β-steroids, aromatic aldehydes, alcohols, sesquiterpenes, naphthalene, stilbene derivatives of benzopyran, benzophenone, caffeic acid, cinnamic acid derivatives, and benzoic acid.[15-17] Propolis exhibits properties such as anti-inflammatory, antioxidant, antimicrobial, antiviral, antiparasitic, anesthetic, and that of a free-radical scavenger.[14]

The use of a varnish, as a treatment modality for DH, is scarce in the literature, making this study innovative, and the concept of a naturally derived varnish adds further to the innovation. Propolis has been used in the form of gels to treat DH, but to the best of our knowledge, no studies have been reported so far which have made use of a propolis varnish for treating DH. Thus, the aim of this study was to assess the effect of a propolis varnish on dentinal tubule occlusion, hence aiding in the treatment of DH.

**Materials and Methods**

This study was carried out in the Department of Periodontology of our hospital following approval from the Ethical Committee. Propolis varnish was prepared at Skanda Life Sciences Laboratory (Bangalore, India). For preparing 15% extract of propolis varnish, 6 g of Forever Bee Propolis® tablets were suspended in 40 mL of ethanol for 4 h at room temperature before it was extracted on a magnetic stirrer. The extract was filtered through Whatmann No. 1 filter paper and was used to prepare the varnish. In fact, we have applied for a patent as the composition and preparation of the varnish is unique. The composition of the varnish,[18] is given in Table 1 while Figure 1 shows the tablets used to prepare the varnish.

In all, 20 freshly extracted teeth, free from caries or any previous conservative or endodontic treatment, were used in this study. They were stored in formalin and used within 1 month of extraction.[19] Crowns were sectioned with a diamond disc, perpendicular to the long axis of the roots to create dentin discs from mid-coronal dentin. Each tooth was its own control. Twenty discs were obtained from each half of the teeth, which were then polished to a thickness of 1 mm. Discs, free of enamel and pulp horns, were stored in saline until scanning electron microscope (SEM) analysis. Discs were divided equally into two groups: group I (test) and group II (control) with 20 discs in each group. In both the groups, the discs were treated with 37% phosphoric acid (etchant) for 30 s, followed by washing with water for 30 s (pretreatment). Then the discs were coated with propolis varnish in the test group (posttreatment for test group) using a thin bristled brush and kept for 10 min before washing with water. The discs in control group were kept in saline (posttreatment for control group). The discs in both the groups were subjected to SEM analysis.

**SEM analysis**

The samples for SEM analysis were first fastened to a metal support with a ribbon of carbon after which the samples were conditioned in a vacuum desiccator to allow for evaporation. On complete drying, they were sputter-coated with gold (50 nm), which is required for SEM analysis (VEGA3 TESCAN). The photomicrographs were obtained at different magnifications: ×400, ×3000, ×5000, ×7000, and ×10000. Figure 2 shows SEM image of the smear layer under ×400 and ×3000 resolutions, respectively. Figure 3 shows SEM images of the sample after acid etching under ×3000 and ×10,000 resolutions, respectively. Figure 4(a) shows SEM images of the sample after the application of propolis varnish under ×5000 resolution; Figure 4(b) shows enlarged image of the same.

<table>
<thead>
<tr>
<th>Table 1: Composition of varnish</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Propolis Varnish components</strong></td>
</tr>
<tr>
<td>Ethanolic propolis extract (EPE, 15%)</td>
</tr>
<tr>
<td>Carboxy methyl cellulose (CMC, 1%)</td>
</tr>
<tr>
<td>Acetic acid (9%)</td>
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<tr>
<td>Water</td>
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<tr>
<td>Total Volume</td>
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</table>
Table 2: Comparison of mean number of opened dentinal tubules between 02 groups at pre-treatment and post-treatment time intervals using independent Student t-test

<table>
<thead>
<tr>
<th>Time</th>
<th>Groups</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>S.E.M</th>
<th>Mean Diff</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>I</td>
<td>20</td>
<td>160.00</td>
<td>6.97</td>
<td>1.56</td>
<td>3.80</td>
<td>1.530</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
<td>156.20</td>
<td>8.65</td>
<td>1.93</td>
<td>1.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>I</td>
<td>20</td>
<td>61.20</td>
<td>9.10</td>
<td>2.04</td>
<td>-95.45</td>
<td>-33.164</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
<td>156.65</td>
<td>9.10</td>
<td>2.04</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant

Statistical analysis

To assess the reliability of obtained results from SEM analysis, detailed statistical analysis was also carried out using Statistical Package for the Social Science (SPSS version 10.5) (IBM Corporation, Armonk, New York, United States). Independent Student’s t-test was used to perform intragroup comparison of pretreatment and posttreatment values in each group. Student’s paired t-test was used to compare the mean value of open dentinal tubules, before and after application of the saline and propolis varnish between two groups. The level of significance was set at $P < 0.001$.

Results

In this study, 20 freshly extracted teeth, free from caries or any previous conservative or endodontic treatment, were used. The discs were subjected to similar treatment of acid etching in both the groups. The treatment of discs varied after the acid etching stage, where in group II the discs were kept in saline and in group I the discs were subjected to propolis varnish application. The mean number of open dentinal tubules was recorded through SEM images, at two time points, that is, after acid etching (pretreatment) and after saline or propolis application (posttreatment).

The SEM photomicrographs, presented in Figure 2, reveal the occluded dentinal tubules as covered by the smear layer. Acid etching was carried out on the samples to remove this smear layer using 37% orthophosphoric acid. This step is essential as it will open the dentinal tubules for effective application of the varnish. The images in Figure 3 show the open dentinal tubules, free from the smear layer. The SEM images in Figure 4 reveal the extent of dentinal tubule occlusion (partial obliteration) achieved after varnish application.

The data obtained were statistically analyzed using independent Student’s t-test (for intragroup comparison) and Student’s paired t-test (for intergroup comparison). As shown in Table 2, the mean number of open dentinal tubules was $160.00 \pm 6.97$ and $156.20 \pm 8.65$ at pretreatment and $61.20 \pm 9.10$ and $156.65 \pm 9.10$ at posttreatment time intervals in groups I and II, respectively. There was a
significant reduction in the mean number of open tubules in the group I discs which approximated to 61.75%. There was no reduction observed in the mean number of open tubules in group II.

The intergroup comparison performed using Student’s paired t-test as shown in Table 3, which reveals a statistically significant difference in the pretreatment and posttreatment values in group I compared with group II. This comparison is represented as a bar graph in Figure 5. The mean number of open tubules pretreatment and posttreatment in group I and II as well as the reduction in the number of mean open tubules in group II are shown in the form of a linear graph in Figure 6.

**Discussion**

It is evident that an in vivo experiment is the ideal methodology for biological investigation, and in vitro studies can only address limited aspects of any natural system. In this study, the extent of dentinal tubule occlusion after treatment with propolis varnish was assessed by counting the number of open tubules before and after treatment. The results of the statistical analysis showed a significant reduction in the number of open tubules, after application of propolis varnish. The results also verified the fact that tubules were mostly occluded to an approximation of about 61.75%.[20]

Various studies carried out on the properties of propolis for oral applications showed that it possesses an anti-inflammatory action. It is also considered to be a powerhouse of bioactive agents and the bioflavonoids, in particular. It is important to note that flavonoids present as a component of propolis are the main active and reactive agents capable of stimulating reparative dentin formation. This is one of the main factors that enable propolis to reduce dentin permeability efficiently.[14] This action is similar to that observed with OXA GEL, which is also a hypersensitivity agent.[21] Based on the hydrodynamic theory, agents used for the treatment of DH should be capable of occluding the dentinal tubules and prevent nerve sensitivity. Thus, propolis is shown to be capable of obliterating the dentinal tubules, which in turn decreases the permeability of the tubules and thereby reduces the DH.

An in vitro study compared the efficacy of 10% and 30% propolis gel in dentin tubule occlusion and showed that lower concentration is better.[21] Studies also verified the biocompatibility and clinical efficacy of nanohydroxyapatite crystals and propolis gel in treating DH through a visual analog scale to evaluate pain among the patients. They suggested propolis as an effective natural alternative for treating DH.[22] Luca MP et al. (2014) assessed the action, efficacy, and cytotoxicity of propolis varnish using chitosan-based propolis varnish of varying concentrations, namely, 5%, 10%, and 15%. The results stated that all the propolis varnish formulations showed a satisfactory level of antimicrobial activity against cariogenic bacteria and had low cytotoxicity; higher concentrations showed larger inhibition zones of microbes with higher release profile. Furthermore, propolis showed a sustained release effect for 24 h.[19]

Short-term studies also showed propolis as a better alternative in comparison to that of 5% potassium nitrate.

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**Table 3: Comparison of mean number of opened dentinal tubules between pre-treatment and post-treatment time intervals in both groups using Student paired t-test**

<table>
<thead>
<tr>
<th>Time</th>
<th>Groups</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>S.E.M</th>
<th>Mean Diff</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>I</td>
<td>20</td>
<td>160.00</td>
<td>6.97</td>
<td>1.56</td>
<td>98.80</td>
<td>38.759</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
<td>61.20</td>
<td>9.10</td>
<td>1.93</td>
<td>-0.45</td>
<td>1.072</td>
<td>0.30</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>I</td>
<td>20</td>
<td>156.20</td>
<td>8.65</td>
<td>1.93</td>
<td>-0.60</td>
<td>-1.072</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
<td>156.65</td>
<td>9.10</td>
<td>2.04</td>
<td>-0.55</td>
<td>-1.072</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Statistically significant

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**Figure 5: Bar comparison of the mean number of opened dentinal tubules between two groups at pretreatment and posttreatment time intervals**

**Figure 6: Line comparison of the mean number of opened dentinal tubules between pretreatment and posttreatment time intervals in both the groups**
in relieving DH. They have proven that propolis has immediate and sustained effects. Immediate relief was attributed to the tubular sealing action of flavonoids and the sustained effect because of the stability of the products formed. Hussain et al. (2016) evaluated the effect of propolis extracts in reducing postbleaching DH in patients. The results of the study confirmed that patients who received propolis application did not experience any DH after bleaching in comparison to control group.

**Conclusion**

Our study critically examined the use of propolis varnish as a natural treatment modality for treating DH. While the composition of varnish used in the study is unique, the efficacy of treatment is established using SEM analysis of high resolution. One of the limitations of this study is that the in vitro oral cavity conditions could not be simulated. The absence of an acid challenge to check for dentinal tubule occlusion after exposure to an acid also contributes to one of the limitations of the study. Based on this in vitro experiment carried out, it is shown that propolis varnish can be used for treating DH, effectively. It not only provides a herbal solution to sensitivity problems but also provides an acceptable alternative to patients who are apprehensive toward surgical approach. Future in vivo studies, comparing propolis varnish to other available varnishes or desensitizing products, can highlight its feasibility in clinical practices.

**Acknowledgement**

The authors are thankful to Skanda Life Sciences Laboratory, Bangalore, India, for the preparation of propolis varnish.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**