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COMPARATIVE EVALUATION OF FOUR HERBAL DESENSITIZING TOOTHPASTE ON DENTINAL TUBULE OCCLUSION- A SCANNING ELECTRON MICROSCOPE ANALYSIS

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ABSTRACT

Background: Dentin hypersensitivity (DH) is a common clinical condition characterized by short, sharp pain arising from exposed dentin in response to thermal, tactile, osmotic, evaporative, or chemical stimuli. Occlusion of exposed dentinal tubules is considered one of the most effective approaches for reducing dentin hypersensitivity. Recently, herbal desensitizing toothpastes have gained popularity because of their natural ingredients and favorable safety profile. However, scientific evidence comparing commercially available herbal formulations remains limited.

Aim: To evaluate and compare the effectiveness of four commercially available desensitizing toothpastes on dentinal tubule occlusion using scanning electron microscopy (SEM).

Materials and Methods: An in vitro experimental study was conducted on 52 freshly extracted human teeth. Standardized dentin specimens measuring approximately 5 × 5 × 3 mm were prepared, polished, and treated with 17% EDTA to remove the smear layer. The specimens were randomly allocated into four groups (n = 13): Group I – Himalaya Sensitive Toothpaste; Group II – Purexa Sensitive Toothpaste; Group III – Sensora Herbal Sensitivity Relief Toothpaste; and Group IV – Sensodyne Sensitive Toothpaste. Brushing was performed using a customized powered toothbrush for 2 minutes twice daily for 14 consecutive days. Following treatment, specimens were sputter-coated with platinum and examined under SEM at ×2000 magnification. Dentinal tubules were categorized as completely occluded, partially occluded, or non-occluded. Statistical analysis was performed using SPSS version 26.0. Since the data were not normally distributed (Shapiro–Wilk test), Kruskal–Wallis and Mann–Whitney U tests were applied. Statistical significance was set at $p < 0.05$.

Results: Group I demonstrated the highest mean number of completely occluded tubules (147.08 ± 65.62), followed by Group IV (135.92 ± 95.27), Group III (129.00 ± 93.07), and Group II (105.15 ± 51.32). However, the difference among groups was not statistically significant ($p = 0.432$). Group I also showed the highest mean number of partially occluded tubules (94.31 ± 49.40), while Group III exhibited the lowest mean number of non-occluded tubules (29.31 ± 23.82). No statistically significant differences were observed among the groups for partially occluded ($p = 0.071$) or non-occluded tubules ($p = 0.386$). Overall, all four toothpastes demonstrated comparable dentinal tubule occlusion.

Conclusion: Within the limitations of this in vitro study, all four commercially available desensitizing toothpastes exhibited comparable efficacy in achieving dentinal tubule occlusion. Although numerical differences were observed, none reached statistical significance. The findings support the use of herbal-based desensitizing formulations as effective alternatives for managing dentin hypersensitivity through dentinal tubule occlusion.

KEYWORDS: Dentin hypersensitivity; Dentinal tubule occlusion; Herbal toothpaste; Scanning electron microscopy; Desensitizing dentifrice; SEM.

INTRODUCTION

Dentin hypersensitivity (DH) is characterized by short, sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic, or chemical stimuli, which cannot be ascribed to any other dental defect or pathology[1] This chronic condition manifests as acute, transient pain in non-carious teeth, particularly when exposed to thermal stimuli such as hot or cold temperatures, chemical stimuli including acidic, sweet, or salty substances, and mechanical stimuli resulting from the exposure of dentinal tubules.[1] The epidemiology of dentin hypersensitivity demonstrates considerable variability across different populations and study methodologies. Systematic review evidence indicates a best global prevalence estimate of 11.5% (95% CI: 11.3%-11.7%), with reported prevalence rates ranging from as low as 1.3% to as high as 92.1% depending on study design, participant selection criteria, and diagnostic approaches.[2] This heterogeneity can be explained by differences in the type of study participants, age ranges studied, recruitment strategies, and number of study sites involved[2] When analyzing affected populations within dental practices, higher prevalence rates (ranging from 30-57%) are observed in convenience samples, particularly among patients seeking specialist dental care.[3] The age of peak

incidence is reported between 30 and 40 years, with epidemiological surveys indicating a slightly elevated occurrence among females compared to males, though these differences are not always statistically significant.[4] Dentin hypersensitivity (DH) is one of the commonly encountered clinical conditions in dental practice, affecting about 4-74% of the population[5] Despite its widespread prevalence and significant impact on quality of life, dentin hypersensitivity remains an underdiagnosed and undertreated condition, primarily because patients often consider the discomfort as a normal consequence of dental procedures or aging. [6]

Dentin hypersensitivity can affect any tooth surface, though the buccal cervical region of canines and premolars demonstrates the highest predilection.[7] The etiology of dentin exposure involves multiple contributing factors resulting in loss of protective hard or soft tissues. Loss of enamel occurs through various mechanical mechanisms including abrasion from abrasive toothbrushing techniques, attrition from mastication, and abfraction from occlusal stress concentration at the cervical region.[8] The coronal cementum layer, which naturally covers the cervical and radicular dentin, is an extremely thin layer measuring only 16-60 micrometers in thickness and is therefore easily removed by natural tooth wear processes.[9] Gingival recession represents the most common etiological factor leading to dentin exposure, with epidemiological surveys revealing that 60-90% of the adult Western European population exhibits some degree of gingival recession.[10] Recent systematic analysis has identified supra- and sub-gingival calculus, low width and thickness of keratinized gingiva, traumatic tooth brushing techniques, and smoking as the most significant etiologic factors associated with gingival recession. Other contributing factors include thin alveolar bone anatomy (alveolar dehiscence and fenestration), periodontal disease, occlusal loading, aggressive brushing habits, and periodontal disease management outcomes. [10] When enamel and cementum are lost or damaged, the underlying dentin layer becomes exposed to the oral cavity and environment, rendering approximately 10,000-15,000 open dentinal tubules per square millimeter patent and accessible to external stimuli.[11] These dentinal tubules are small cylindrical structures measuring 1-2 micrometers in diameter that extend from the dentinoenamel junction (or cementoenamel junction) to the pulp chamber, and they contain odontoblastic processes and are partially filled with tissue fluid.[12] The tubular nature of dentin and the patency of these tubules is a critical structural factor in the pathophysiology of dentin hypersensitivity, as open tubules serve as pathways for the transport of bacterial elements from the oral cavity toward the pulp, potentially leading to inflammatory pulpal responses and increasing sensitivity perception.[12]

Among several theories which explain the mechanism underlying dentinal hypersensitivity, the hydrodynamic theory, first given by Gysi in 1900 and subsequently elaborated by Brännström and Aström in 1966, remains the most widely accepted and scientifically supported explanation. [13,14] According to this theory, fluid within the dentinal tubules can flow either inward or outward based on pressure and osmotic gradients in the surrounding tissue. [14] External stimuli such as thermal, evaporation, tactile, osmotic, or chemical changes induce fluid movement within the tubules, which mechanically activates C-fiber nerve endings located at the pulp-dentin interface, thereby producing the characteristic sharp pain associated with dentin hypersensitivity. [14] The intensity of pain sensation is directly proportional to both the diameter and number of exposed and patent dentinal tubules, as well as the magnitude of fluid flow through these tubules. [15]This mechanistic understanding forms the scientific rationale for desensitizing treatment strategies that aim to reduce fluid conductance through dentinal tubules. [16]

The management of dentin hypersensitivity involves a two-pronged approach, firstly, addressing the underlying etiological causes such as improving oral hygiene techniques, controlling aggressive brushing, managing periodontal disease, and treating acid erosion; and second is implementing desensitizing agents or procedures to directly alleviate patients' discomfort. [17]Therapeutic desensitizing agents work through two primary mechanisms: physical occlusion of exposed dentinal tubules to reduce fluid flow, or direct desensitizing effects on pulpal nerve fibers through depolarization of the nerve membrane. [16] The main characteristics of an ideal desensitizing agent include rapid and sustained pain relief, ease of application, affordability, minimal damage to pulp tissue, reduced potential for tooth staining or discoloration, good patient tolerance, and resistance to mechanical and acid challenge. [17]

Treatment modalities for dentin hypersensitivity can be broadly categorized into at- home and in-office methods. At-home procedures utilize active ingredients delivered through toothpastes, chewing gums, gels, or mouthwashes that patients apply independently on a daily basis. [18] In-office therapy encompasses more intensive professional interventions including the application of varnishes, adhesive systems, iontophoresis, diode laser therapy, and restorations using composite resin or glass ionomer cement. [18] For many patients with mild to-moderate sensitivity, at-home desensitizing toothpastes represent the first-line treatment due to their accessibility, convenience, and cost-effectiveness. [19] The contemporary market offers numerous dentifrice formulations containing diverse chemical ingredients that claim to reduce dentin hypersensitivity. A comprehensive analysis of 138 randomized controlled trials examining toothpaste formulations for treating dentin hypersensitivity identified 368 different formulations, with a significant increase in available options noted since 1968, particularly after 2010 when product innovation accelerated substantially. [20] The analysis identified potassium compounds (potassium nitrate, potassium chloride, potassium citrate), calcium sodium phosphosilicate (NovaMin), strontium compounds, and arginine as commonly tested active ingredients in conventional formulations. [20]

Clinical efficacy studies have demonstrated varying degrees of effectiveness among different active ingredients. NovaMin-containing toothpaste (calcium sodium phosphosilicate) has demonstrated superior long-term

improvement in reducing dentinal hypersensitivity compared to potassium-based compounds, achieving approximately 87-91% sensitivity reduction after 12 weeks. [21] Arginine-containing formulations (8% concentration) have shown superior reduction in dentin hypersensitivity compared to potassium salt and some herbal toothpastes over a four- week period. [22] However, despite the availability of numerous conventional products, no single method has proven to completely eliminate dentin hypersensitivity and provide complete, lasting relief to all patients, establishing a continued need for novel desensitizing formulations. [23]

In recent years, herbal agents have been gaining significant popularity in the management of dentin hypersensitivity, driven by consumer preference for natural products free from synthetic chemicals. This market trend is supported by epidemiological data showing that the global herbal toothpaste market has expanded from USD 2.48 billion in 2024 to a projected USD 8.4 billion by 2034, representing a compound growth rate annually of 3.5-8.9%. [24]

Herbal toothpastes claim to reduce sensitivity through two primary mechanisms: blocking the pain transmission pathway through depolarization effects on nerve fibers, or physically occluding the dentinal tubules through mineral deposition and crystal formation. [25] These formulations contain natural ingredients which are free from artificial colors, flavors, and preservatives typically found in synthetic products, thereby potentially offering enhanced safety profiles for oral health applications. [26] Several herbal ingredients have demonstrated pharmacological promise in managing dentin hypersensitivity. Phytocomplexes obtained from rhubarb stalks (Rheum rhaponticum) and spinach leaves (Spinacia oleracea) have been found to be particularly effective, reducing dentin hypersensitivity by occluding dentinal tubules through the formation of acid-resistant calcium oxalate crystals. [26]The mechanism of action involves soluble oxalates and oxalic acid present in these plant extracts forming insoluble calcium oxalate crystals (weddellite; $CaC_2O_4 \cdot 2H_2O$) approximately 1-2 micrometers in size when the extracts contact dentin containing calcium ions. [26]These microcrystals deposit both at the orifices of dentinal tubules and penetrate partially into the tubular lumen, resulting in occlusion of tubule openings and reduction of fluid conductance. [27]

Importantly, in vitro studies using scanning electron microscopy confirmed that these calcium oxalate crystals remain intact even after acid challenge (37% orthophosphoric acid), demonstrating superior acid resistance compared to many conventional desensitizing agents. [27]Clove (Syzygium aromaticum), another commonly used herbal ingredient, contains the volatile compound eugenol, which produces an obtundant (numbing) effect that desensitizes nerve endings and thereby reduces pain perception through depolarization mechanisms. [28]Propolis, a natural resinous substance produced by honeybees, contains phenolic compounds, flavonoids, and caffeic acid derivatives that have been demonstrated to possess potent antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties. [29]

Research evaluating propolis-based herbal toothpaste formulations has demonstrated effectiveness in blocking dentinal tubules, with propolis-treated specimens showing complete or near complete occlusion of open tubules compared to control groups and achieving comparable efficacy to conventional desensitizing agents containing NovaMin. [30] Recent comparative clinical studies have provided encouraging evidence for herbal alternatives. In a 2023 four-week clinical trial, a herbal toothpaste formulation containing propolis, guava leaves extract, bentonite clay, hemp extract, peppermint extract, tea tree extract, clove oil, and grapeseed extract (Bentodent) demonstrated superior efficacy in reduction of dentin hypersensitivity compared to both potassium nitrate-containing toothpaste and was comparable to calcium sodium phosphosilicate (NovaMin)-containing toothpaste. [31] The multiple herbal ingredients work synergistically, with bentonite clay and propolis occluding tubules, guava extract providing rapid pain relief, and essential oils (peppermint, tea tree) offering antimicrobial and anti-inflammatory effects. [31] Another recent study confirmed that propolis-based herbal toothpaste is as effective as 5% sodium fluoride varnish in occluding human dentin tubules. [32]

Despite the growing popularity and consumer adoption of herbal toothpastes for managing dentin hypersensitivity, there exists very limited scientific literature specifically examining the comparative effectiveness of multiple herbal desensitizing formulations using standardized methodology. [33] Most available in vitro studies compare single herbal products against conventional chemical-based desensitizing agents or evaluate limited herbal formulations using varying experimental protocols, making direct comparisons difficult. [33] Furthermore, while some clinical studies have assessed symptom relief through subjective pain measurements, relatively few investigations have employed scanning electron microscopy to directly visualize, qualitatively assess, and quantify the extent and depth of dentinal tubule occlusion achieved by herbal toothpastes. [34]

The existing literature demonstrates significant methodological variation regarding specimen preparation, treatment application protocols (brushing duration and frequency), acid challenge parameters, and scanning electron microscopic analysis criteria, limiting the ability to perform meta-analysis or systematic comparison of herbal formulations. [35] Additionally, most contemporary studies focus on individual herbal ingredients (such as propolis alone or spinach alone) rather than evaluating commercially available herbal toothpaste formulations that typically contain multiple active ingredients and synergistic plant compounds. [35]Given the increasing consumer preference for natural products and the potential advantages of herbal formulations in terms of biocompatibility, safety profile, and lower toxicity compared to synthetic agents, there is a pressing need for rigorous, systematically designed scientific evaluation of commercially available herbal desensitizing toothpastes. [36] Understanding the comparative effectiveness of different commercially available herbal formulations in achieving dentinal tubule

occlusion, and the depth and durability of this occlusion when subjected to acid challenge, would provide valuable evidence- based guidance for clinicians and patients in selecting appropriate natural treatment options for dentin hypersensitivity management. [36]Such data would also establish scientific credibility for herbal products in the competitive dental care market and support informed clinical decision making.[37]

AIM AND OBJECTIVES

Aim of the Study:

To evaluate and compare the effectiveness of four commercially available herbal desensitizing toothpastes on dentinal tubule occlusion using scanning electron microscope (SEM) analysis.

Objectives of the study:

•To evaluate the effectiveness of Sensora sensitivity toothpaste (containing 5% potassium nitrate in natural forms including spinach, clove, thymol, and camphor) in occluding dentinal tubules.
•To evaluate the effectiveness of Purexa sensitive toothpaste (containing spinach, rhubarb, arnica, and licorice) in occluding dentinal tubules.
•To evaluate the effectiveness of Himalaya Sensitive toothpaste (containing spinach, almond shell extract, and menthol) in occluding dentinal tubules.
•To evaluate the effectiveness of Sensodyne Sensitive toothpaste (containing 5% sodium fluoride with eucalyptus and fennel extracts) in occluding dentinal tubules.
•To compare the effectiveness of these four herbal desensitizing toothpastes in achieving dentinal tubule occlusion and to determine which formulation demonstrates superior efficacy.

MATERIALS AND METHODOLOGY

SOURCE OF DATA:

Study Design
The present study was an in-vitro experimental study designed to evaluate and compare the effectiveness of four herbal desensitizing toothpastes on dentinal tubule occlusion using Scanning Electron Microscopy (SEM).
Study Setting
The study was conducted in the Department of Periodontology, Bharati Vidyapeeth
(Deemed to be University) Dental College and Hospital, Navi Mumbai. Scanning
electron microscopic evaluation of the samples was carried out at Indian Institute of Technology, Mumbai.

Sample Selection

A total of 52 freshly extracted human teeth extracted for orthodontic or periodontal reasons were collected from the Department of Oral Surgery, Department of Orthodontics, Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, and selected private dental clinics.

METHOD OF SELECTION OF STUDY SUBJECTS:

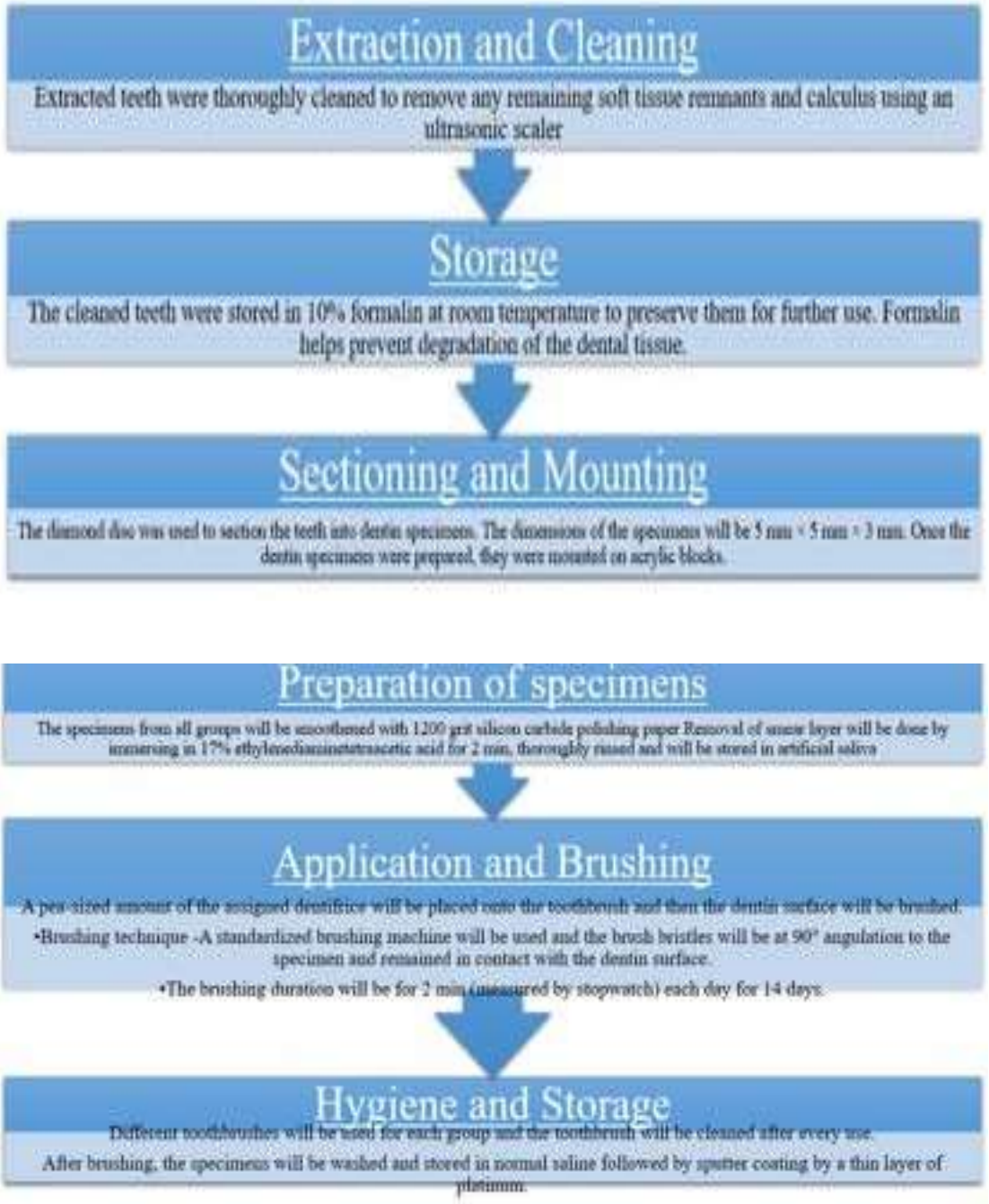
i. Inclusion criteria:
- Human teeth which will be extracted due to orthodontic purpose.
- Teeth with intact crown and root surfaces.
- Teeth unaltered by the extraction procedure

ii. Exclusion criteria:
- Teeth with grossly decayed crown and root caries
- Teeth with restorations and fractures on the tooth surface.
- Teeth with any developmental anomalies.
- Teeth with fluorosis.

ETHICAL CONSIDERATION:

The study protocol was approved by the Scientific Review Committee and Institutional Ethical Committee of Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Navi Mumbai. As the study was conducted on extracted human teeth, no intervention was performed on patients, and informed consent was not required.

STUDY DESIGN AND METHODOLOGY



ARMAMENTARIUM/EQUIPMENTS:

- Surgical gloves
- Sterile gauze and cotton
- Disposable head cap
- Mouth mask
- Mouth mirror
- Kidney tray
- Straight probe
- UNC-15 periodontal probe
- Tweezer
- Ultrasonic scaler
- 10% Formalin
- Acrylic blocks
- Diamond disc
- 1200 grit silicon carbide polishing paper
- 17% ethylenediaminetetraacetic acid

- Customised power toothbrush
- Sensora Herbal Sensitivity Relief Toothpaste
- Purexa Sensitive Toothpaste
- Himalaya Sensitive Toothpaste
- Sensodyne Sensitive Toothpaste
- Sample container
- Normal Saline

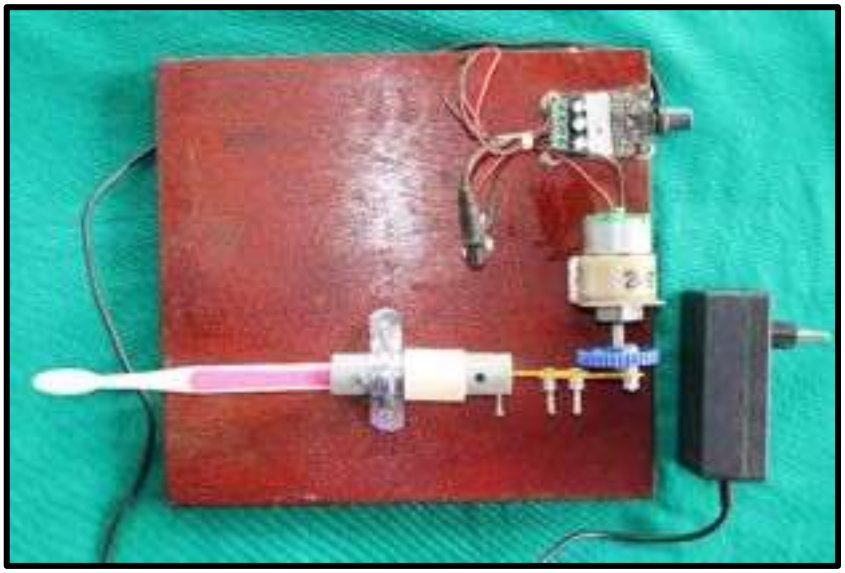


Figure 1 - Standardised Power Toothbrush

Figure 2- Preparation of dentin discs and Mounting the teeth on acrylic blocks for sectioning

PROCEDURE:
Cleaning and Storage of Samples
•Immediately after extraction, the teeth were thoroughly cleaned to remove soft tissue remnants and calculus using an ultrasonic scaler.
•The cleaned teeth were stored in 10% formalin at room temperature until further processing to prevent degradation of dental tissues.

Preparation of Dentin Specimens
•The teeth were sectioned using a diamond disc to obtain dentin specimens measuring approximately 5 mm × 5 mm × 3 mm. The dentin specimens were then mounted on acrylic blocks.
•The mounted specimens were smoothened using 1200-grit silicon carbide polishing paper to obtain a standardized flat dentin surface.

Removal of Smear Layer
•For removal of smear layer, all specimens were immersed in 17% EDTA for 2 minutes, thoroughly rinsed with distilled water, and stored in normal saline.

Grouping of Samples

- The 52 dentin specimens were randomly divided into four groups of 13 specimens each:

a) Group 1: Samples treated with Himalaya Sensitive Toothpaste
b) Group 2: Samples treated with Purexa Sensitive Toothpaste
c) Group 3: Samples treated with Sensora Herbal Sensitivity Relief Toothpaste
d) Group 4: Samples treated with Sensodyne Sensitive Toothpaste

Application of Dentifrice

•Each specimen was mounted onto acrylic blocks so as to be brushed by the customized powered toothbrush in a standardized position.
•A pea-sized amount of the designated dentifrice was placed on the dentin surface of each specimen and the brush bristles were positioned at 90° angulation to the dentin surface.
•The customized brushing model was fabricated to deliver a uniform force of about 1.7N. It was fabricated to produce unidirectional motion with 170 strokes per minute.
•The toothbrushes had a standardized uniform diameter of 0.2 mm with soft bristles.
•Brushing was performed for 2 minutes per day twice daily for a period of 14 consecutive days.
•Separate toothbrushes having the same specification were used for each group to avoid cross-contamination.
•After brushing, the specimens were thoroughly washed and stored in normal saline until SEM evaluation.

Scanning Electron Microscopy Evaluation

•After completion of the brushing protocol, the specimens were prepared for SEM analysis.
•The samples were dried and sputter-coated with a thin layer of platinum and examined under a scanning electron microscope.
•SEM evaluation was carried out at a standardized magnification of 2000x. For each specimen, a single representative SEM field was analyzed.
•Dentinal tubules were assessed and categorized as:
a)Completely occluded
b)Partially occluded
c)Not occluded
•Counting of dentinal tubules was performed manually from the SEM micrographs. The total number of dentinal tubules was also recorded for each specimen.
•Variables Studied
a)Number of completely occluded dentinal tubules
b)Number of partially occluded dentinal tubules
c)Number of non-occluded dentinal tubules

Figure 3- UV radiation for drying of samples

Figure 4– Platinum sputter coating

Figure 5 – Inside the SEM Machine

Figure 6– SEM Machine

STUDY HYPOTHESIS:

•Null hypothesis (H0):There will be no difference in the efficacy of dentin tubule occlusion in group A, group B and group C and group D

H0 = μ1 = μ2 = μ3= μ4

Where,

μ1 = Group A μ2 = Group B μ3 = Group C μ4 = Group D

•Alternate hypothesis (H1): There will be difference in the efficacy of dentin tubule occlusion in group A, group B and group C and group D

H1 = μ1 ≠ μ2 ≠ μ3≠ μ4

SAMPLE SIZE ESTIMATION:

Sample size was determined using the mean and standard deviation values obtained from previously published literature evaluating dentinal tubule occlusion using scanning electron microscopy.

The sample size was calculated using the following formula:

Where:

- 1−/2= 1.96 (Type I error of 5%)
- 1−= 0.84 (Type II error of 20%, power of 80%)
- = pooled standard deviation (0.965)
- = minimum expected difference between groups (1.09)

Substituting the values:

Thus, a minimum of 13 samples per group was required to complete the study. Accordingly, a total of 52 samples were included and equally distributed among the four study groups.

METHODS OF DATA ANALYSIS -

•All data were entered into Microsoft Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States) and subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp.)

•Normality of numerical data was assessed using the Shapiro–Wilk test, which showed that the data did not follow a normal distribution. Hence, non-parametric tests were used for statistical comparisons.

•Intergroup comparison among more than two groups was carried out using the Kruskal Wallis test, followed by pairwise comparison using the Mann– Whitney U test.

For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

* = statistically significant difference ($p<0.05$)

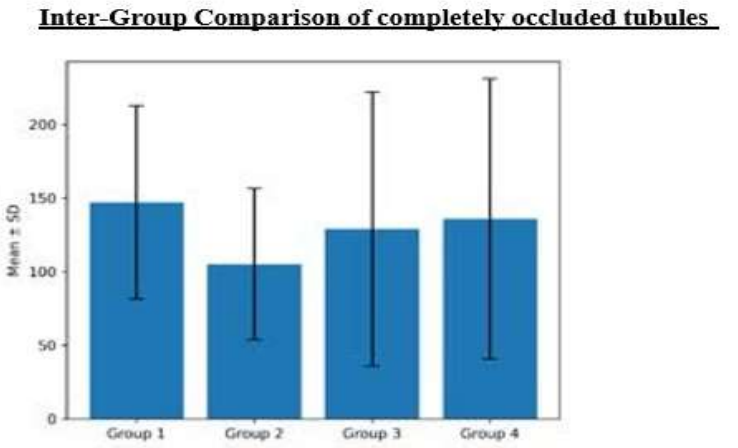
** = statistically highly significant difference ($p<0.01$) # = non significant difference ($p>0.05$)

RESULTS

Group		N	Mean	Std. Deviation	Median	Mean Rank	Chi square value	p value of Kruskal-Wallis Test
Completely Occluded	1	13	147.08	65.622	140	31.96	2.751	0.432#
	2	13	105.15	51.322	96	22.77		
	3	13	129.00	93.072	93	24.35		
	4	13	135.92	95.268	94	26.92		
Partially Occluded	1	13	94.31	49.478	77	33.96	7.018	0.071#
	2	13	64.15	43.871	56	23.12		
	3	13	56.92	39.180	44	19.54		
	4	13	78.77	45.633	65	29.38		
No Occlusion	1	13	46.08	35.587	30	29.23	3.038	0.386#
	2	13	44.08	34.784	24	25.92		
	3	13	29.31	23.817	20	20.77		
	4	13	47.00	32.519	40	30.08		

Table 1- Inter group comparison of Completely Occluded, Partially Occluded, No Occlusion in 1, 2, 3 and 4 groups .

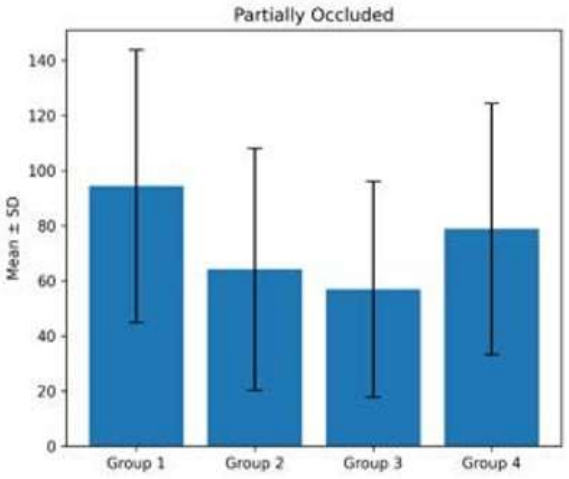
There was a statistically non significant difference seen for the values between the groups ($p>0.05$) for all values in 1, 2, 3 and 4 groups



Graph 1 - Bar chart showing the Mean ± SD of Completely Occluded tubules per treatment group

The mean number of completely occluded tubules showed variation across the four treatment groups.
Group 1 (Himalaya Sensitive Toothpaste) demonstrated the highest mean occlusion with 147.08 ± 65.62 completely occluded tubules per specimen. Group 2 (Purexa Sensitive Toothpaste) showed the lowest mean of 105.15 ± 51.32 completely occluded tubules.
Group 3 (Sensora Herbal Sensitivity Relief Toothpaste) showed mean completely occluded tubules of 129.00 ± 93.07.
Group 4 (Sensodyne Sensitive Toothpaste) showed a total mean occluded tubules of 135.92 ± 95.27.
The Kruskal-Wallis test for completely occluded tubules revealed no statistically significant difference among the four treatment groups ($p = 0.432$, $p>0.05$). Although numerical differences existed in mean values, with Group 1 demonstrating numerically higher mean occlusion (147.08) compared to other groups, these differences did not achieve statistical significance. This suggests that all four herbal desensitizing toothpastes possessed comparable efficacy in achieving complete dentinal tubule occlusion.

Comparison of Partially Occluded Tubules



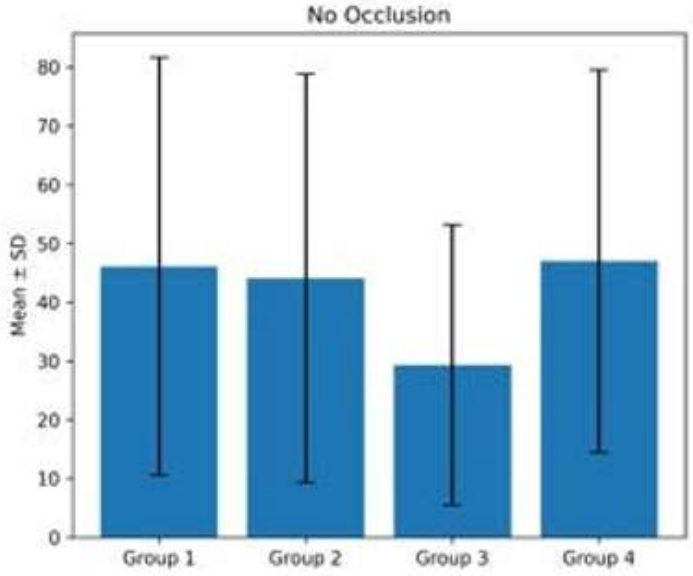
Graph 2 - Bar chart showing the Mean ± SD of Partially Occluded tubules per treatment group

Graph 2 - Bar chart showing the Mean ± SD of Partially Occluded tubules per treatment group

The mean number of partially occluded tubules exhibited distinct patterns across treatment groups.
Group 1 (Himalaya) demonstrated the highest mean partial occluded tubules with 94.31 ± 49.4.
Mean partially occluded tubules in Group 2 were 64.15 ± 43.8 Mean partially occluded tubules in Group 3 were 56.92 ± 39.1 Mean partially occluded tubules in Group 4 were 78.77 ± 45.6

The Kruskal-Wallis test for partially occluded tubules demonstrated a trend toward significance but not statistically significant ($p = 0.071$, $p>0.05$, approaching but not meeting a significance threshold of $p<0.05$). The near-significant p-value suggests that treatment groups may have differed in their ability to achieve partial tubule occlusion, though the difference did not reach statistical significance with the current sample size (n=13 per group).

Comparison of Non-Occluded Tubules (No Occlusion)



Graph 3 - Bar chart showing the Mean ± SD of tubules with No Occlusion per treatment group

The mean number of non-occluded tubules (those remaining patent and not occluded by treatment) demonstrated important differences between groups.

Group 1 (Himalaya) showed mean of 46.08 ± 35.5 of non-occluded tubules. Group 2 (Purexa) showed mean of 44.08 ± 34.78 of non-occluded tubules. Group 3 (Sensora Herbal Sensitivity Relief Toothpaste) achieved the lowest mean of 29.31 ± 23.82 non-occluded tubules per specimen, indicating the most comprehensive tubule occlusion relative to the total number of exposed tubules in that group. Group 4 (Sensodyne) demonstrated the highest mean of 47.00 ± 32.52 non-occluded tubules.

The Kruskal-Wallis test for non-occluded (patent) tubules showed no statistically significant difference among treatment groups $p = 0.386$, $p>0.05$). All treatment groups achieved broadly comparable reductions in the number of patent tubules exposed to external stimuli.

Normality Testing and Statistical Test Selection

		Shapiro-Wilk		
	Group	Statistic	N	p value
Completely Occluded	1	.952	13	.629
	2	.880	13	.071
	3	.874	13	.058
	4	.713	13	.001
Partially Occluded	1	.919	13	.246
	2	.901	13	.138
	3	.745	13	.002
	4	.841	13	.022
No Occlusion	1	.815	13	.010
	2	.854	13	.032
	3	.734	13	.001
	4	.896	13	.118

Table 2- Tests of Normality

p<0.05 indicates normality not followed
Prior to conducting parametric statistical analyses, the normality of data distribution was assessed using the Shapiro-Wilk test to determine the appropriateness of parametric versus non parametric statistical procedures. The results of normality testing indicated that the data did not follow a normal (Gaussian) distribution for multiple measured parameters across the treatment groups.
Specifically:
•Completely Occluded Tubules: Group 4 demonstrated failure of normality assumption (p=0.001, p<0.05)
•Partially Occluded Tubules: Groups 3 (p=0.002, p<0.05) and Group 4
(p=0.022, p<0.05) failed the normality test
•No Occlusion Tubules: Groups 1 (p=0.010, p<0.05), 2 (p=0.032, p<0.05), and 3 (p=0.001, p<0.05) failed the normality test
Due to the failure of multiple parameters to meet the normality assumption, non- parametric statistical tests were employed for inter-group comparisons rather than parametric tests. This approach is more appropriate and conservative when data distributions deviate from normality, reducing the risk of Type I and Type II statistical errors.

Pairwise Comparisons: Mann-Whitney U Test Results
Following the overall Kruskal-Wallis ANOVA tests, post-hoc pairwise comparisons were conducted using the Mann-Whitney U test (non- parametric equivalent of independent samples t-test) with Bonferroni correction applied to control for multiple comparisons and
maintain family-wise error rate.

	Mann-Whitney U value	**Z value**	**p value of Mann-Whitney U test**
Completely Occluded	49.500	-1.797	0.072#
Partially Occluded	52.500	-1.642	0.101#
No Occlusion	76.500	-0.411	0.681#

Table 3- Inter group Pair wise comparison of Group 1vs2 using Mann-Whitney U test

Mann-Whitney U test; *indicates statistically significant p<0.05; # indicates statistically non significant p>0.05. There was a statistically non significant difference seen for the values between the groups (p<0.01) for all values.

	Mann-Whitney U value	**Z value**	**p value of Mann-Whitney U test**
Completely Occluded	64.500	-1.026	0.305#
Partially Occluded	36.500	-2.464	0.014*
No Occlusion	55.000	-1.521	0.128#

Table 4- Inter group Pair wise comparison of Group 1vs3 using Mann-Whitney U test

Mann-Whitney U test; *indicates statistically significant p<0.05; # indicates statistically non significant p>0.05. There was a statistically non significant difference seen for the values between the groups (p<0.01) for all values; while statistically significant difference seen for the values between the groups (p<0.05) for Partially Occluded Tubules.

	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Completely Occluded	68.500	-0.821	0.412#
Partially Occluded	67.500	-0.872	0.383#
No Occlusion	82.500	-0.103	0.918#

Table 5- Inter group Pair wise comparison of Group 1vs4 using Mann-Whitney U test

Mann-Whitney U test; # indicates statistically non significant p>0.05.
There was a statistically non significant difference seen for the values between the groups (p<0.01) for all values in group 1vs4

	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Completely Occluded	83.000	-0.077	0.939#
Partially Occluded	76.000	-0.436	0.663#
No Occlusion	72.000	-0.643	0.520#

Table 6- Inter group Pair wise comparison of Group 2vs3 using Mann-Whitney U test

Mann-Whitney U test; # indicates statistically non significant p>0.05.
There was a statistically non significant difference seen for the values between the groups (p<0.01) for all values in group 2vs3

	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Completely Occluded	72.500	-0.617	0.537#
Partially Occluded	64.000	-1.051	0.293#
No Occlusion	72.500	-0.616	0.538#

Table 7- Inter group Pair wise comparison of Group 2vs4 using Mann-Whitney U test

Mann-Whitney U test; # indicates statistically non significant p>0.05.
There was a statistically non significant difference seen for the values between the groups (p<0.01) for all values in group 2vs4

	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Completely Occluded	75.000	-0.487	0.626#
Partially Occluded	50.500	-1.744	0.081#
No Occlusion	52.000	-1.674	0.094#

Table 8- Inter group Pair wise comparison of Group 3vs4 using Mann-Whitney U test

Mann-Whitney U test; # indicates statistically non significant p>0.05.
There was a statistically non significant difference seen for the values between the groups (p<0.01) for all values in group 3vs4

Summary of Statistical Findings
Primary Findings
1.No overall statistically significant difference in completely occluded tubules among the four herbal desensitizing toothpastes (Kruskal-Wallis: p=0.432)
2.Trend toward significance in partially occluded tubules (Kruskal- Wallis: p=0.071), with Group 1 (Himalaya) achieving numerically higher partial occlusion, significantly greater than Group 3 (Mann-Whitney: p=0.014) *
3.No significant difference in non-occluded tubules among treatment groups (Kruskal Wallis: p=0.386)
4.Significant differences in total tubule count between groups, attributed to specimen variation rather than treatment effect
Secondary Findings
•Group 1 (Himalaya Sensitive Toothpaste) demonstrated the highest mean complete occlusion (147.08 ± 65.62 tubules) and partial occlusion (94.31 tubules)
•Group 3 (Sensora Herbal Sensitivity Relief Toothpaste) achieved the lowest mean non-occluded tubules (29.31 ± 23.82), indicating the highest relative proportion of occluded tubules regardless of occlusion category
•Group 2 (Purexa Sensitive Toothpaste) showed the most conservative occlusion pattern with the lowest mean complete occlusion (105.15 ± 51.32) and moderate mean partial occlusion (64.15)

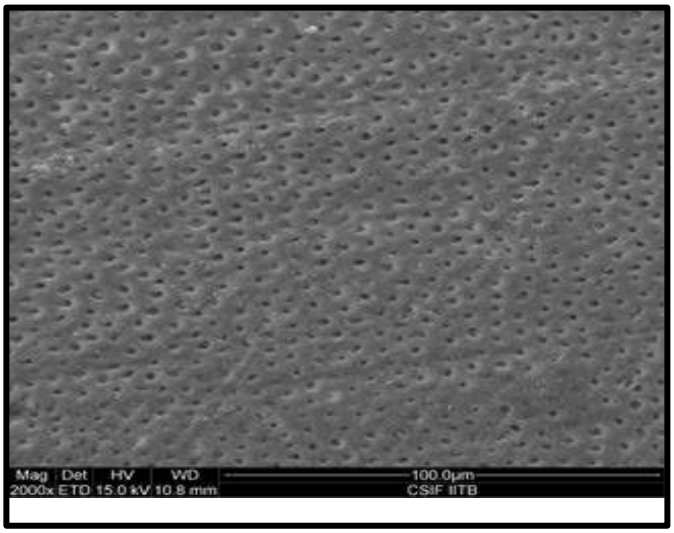


Figure 7- SEM image of specimen treated by Himalaya Sensitive Toothpaste

•Group 4 (Sensodyne Sensitive Toothpaste) demonstrated intermediate performance.
All the four toothpastes gave comparable results as far as tubule occlusion is concerned.
References to Data Sources

All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS version 26.0, IBM Corporation, Armonk, New York, United States) on a Microsoft Windows 11 operating system. Raw data compilation was performed using Microsoft Excel (2019,
Microsoft Redmond Campus, Redmond, Washington, United States). The significance level (α) was set at 0.05 with a statistical power (1-β) of 0.80 (80%), representing a Type II error (β) of 0.20 (20%).
Statistical Significance Notation Key
* = statistically significant difference ($p<0.05$)
** = statistically highly significant difference ($p<0.01$) # = non-significant difference ($p>0.05$

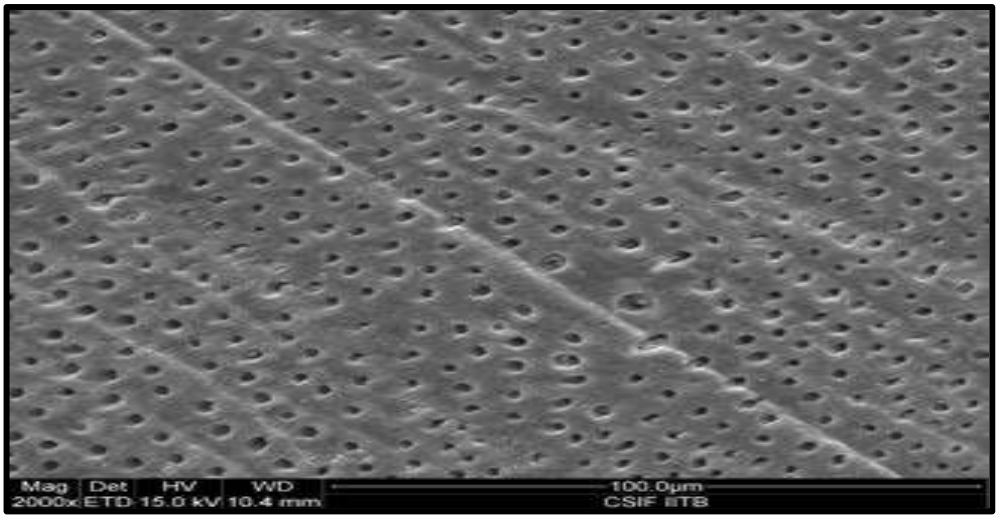


Figure 8– SEM image of specimen treated by Purexa Sensitive Toothpaste

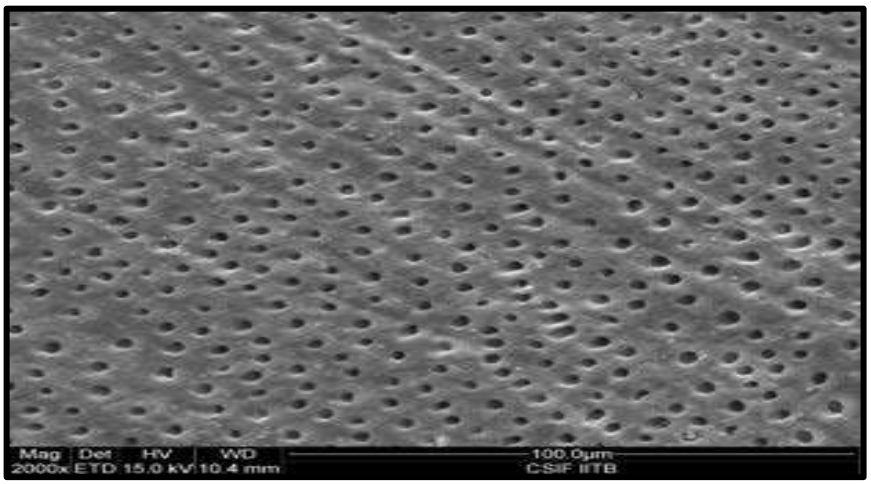


Figure 9 - SEM image of specimen treated by Sensora Sensitive Toothpaste

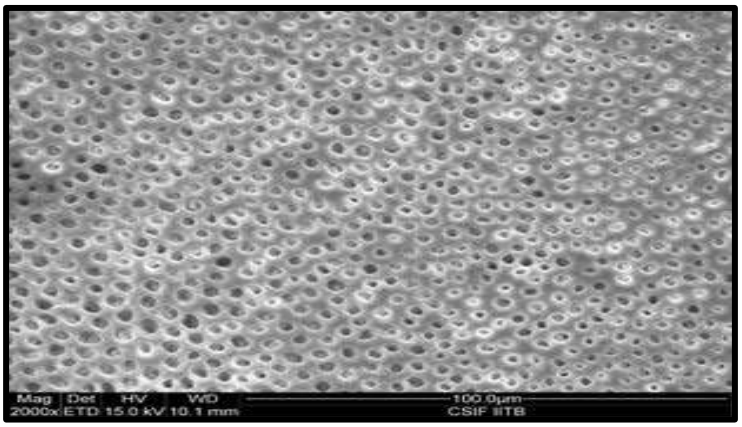


Figure 1 - SEM image of specimen treated by Sensodyne Sensitive Toothpaste

DISCUSSION

Dentin hypersensitivity (DH) represents a significant clinical burden due to its high and widely variable prevalence, with reports ranging from minimal levels in epidemiological surveys to as high as 30–57% in clinical populations. [2,3,5] It predominantly affects individuals in the 3rd and 4th decades of life, thereby impacting a functionally active group and interfering with routine activities such as eating, drinking, and oral hygiene practices.[4] Despite its frequency, DH remains underdiagnosed and undertreated, as patients often perceive it as a normal condition rather than a treatable pathology.[6] The associated behavioral adaptations, including avoidance of proper brushing or certain dietary habits, may further compromise oral health. Although conventional desensitizing agents such as potassium salts, calcium sodium phosphosilicate, and arginine have demonstrated efficacy, none provide complete or consistently long-term relief. [20–23] In this context, there has been a growing shift toward herbal dentifrices, driven by increasing patient preference for natural products and their potential advantages, including multimodal mechanisms of action, improved biocompatibility, and reduced adverse effects.[24–26] Herbal formulations, incorporating agents such as oxalates, eugenol, and propolis, have shown promising results in achieving dentinal tubule occlusion and nerve desensitization, with some evidence suggesting comparable efficacy to conventional agents.[26–32] However, the lack of standardized comparative data on commercially available herbal toothpastes highlights the need for systematic evaluation, forming the basis for the present study.[33–36]

The present in vitro scanning electron microscopic study compared the effectiveness of four commercially available desensitizing toothpastes in achieving dentinal tubule occlusion. A total of 52 dentin specimens obtained from freshly extracted human premolar teeth were randomly allocated into four groups, with 13 specimens in each group. Standardized specimen preparation, controlled brushing protocols, and uniform SEM evaluation were employed to objectively assess dentinal tubule occlusion patterns.[57] The principal finding of this investigation was the absence of a statistically significant difference among the four study groups with respect to completely occluded dentinal tubules (Kruskal–Wallis test, $p = 0.432$). Although numerical variations were observed, these differences did not reach statistical significance, indicating that all four formulations demonstrated comparable efficacy in occluding dentinal tubules under the experimental conditions of the present study. [58] Group 1 (Himalaya Sensitive Toothpaste), containing spinach and rhubarb demonstrated the highest mean number of completely occluded tubules (147.08 ± 65.62), followed by Group 4 (Sensodyne Sensitive Toothpaste: 135.92 ± 95.27), Group 3 (Sensora Herbal Sensitivity Relief Toothpaste: 129.00 ± 93.07), and Group 2 (Purexa Sensitive Toothpaste: 105.15 ± 51.32). [47,48]

When interpreted relative to the total number of tubules, Group 1 exhibited approximately 51.2% completely occluded tubules (147.08 out of 287.46), a value comparable to occlusion percentages reported for herbal formulations in previous SEM-based studies.

Despite these numerical differences, the lack of statistical significance suggests that the mechanism of tubule occlusion is achieved effectively across all formulations, regardless of differences in botanical composition or inclusion of conventional desensitizing agents such as fluoride or potassium nitrate.[59]

The comparable performance observed across all groups can be explained by established principles of dentinal tubule occlusion.[60] Pereira et al. demonstrated that the reduction of dentin permeability is primarily dependent on the presence of deposits within or over dentinal tubules, irrespective of whether the source of deposition is synthetic or natural.[27] Group 1 (Himalaya) contains spinach and rhubarb extracts, both rich in soluble oxalates.[26] Oxalates react with calcium ions present in dentin to form insoluble calcium oxalate crystals, which deposit at tubule orifices and partially within the tubule lumen. These crystals are resistant to acidic dissolution and mechanically stable, making them effective occluding agents.[27] The formulation includes spinach and almond shell extract, the latter containing tannins and polyphenolic compounds capable of protein precipitation and surface sealing. Menthol, while not contributing directly to physical occlusion, may enhance analgesic perception through neural pathways.[28]

This multi-component composition may explain the numerically higher occlusion values observed in this group.[25] Group 2 (Purexa) although demonstrating the lowest numerical values for complete occlusion, still produced substantial tubule blocking. In addition, arnica and licorice may contribute synergistically through astringent, anti-inflammatory, and protective effects, potentially enhancing crystal retention and surface coverage.[61] Group 3 (Sensora Herbal Sensitivity Relief Toothpaste) represents a dual- mechanism formulation, combining 5% potassium nitrate with herbal components such as spinach, clove, thymol, and camphor. Potassium nitrate primarily acts through neural desensitization by depolarizing intradental nerve fibers, while the herbal components contribute to physical tubule occlusion. This group demonstrated the lowest mean number of non-occluded tubules, suggesting more uniform dentin surface coverage. [28] Group 4 (Sensodyne Sensitive Toothpaste), included as a comparative formulation, contains 5% sodium fluoride with eucalyptus and fennel extracts. Fluoride contributes to tubule occlusion through the formation of calcium fluoride and fluorapatite precipitates, while the herbal extracts provide ancillary antimicrobial and anti-inflammatory benefits. Although Group 4 showed a high mean for completely occluded tubules, it also demonstrated the highest numerical mean for non-occluded tubules, indicating greater variability in occlusion patterns. [62] A notable finding across all groups was the high standard deviation values, particularly in Group 4. This variability reflects the inherent biological heterogeneity of dentin, including differences in tubule diameter, density, and orientation even within

similar tooth types.[12,63] Minor variations in specimen preparation, polishing, and smear layer removal may also contribute to inter-specimen variability.[64,65]

The Shapiro–Wilk test revealed non-normal data distribution, justifying the use of non-parametric tests. The Kruskal–Wallis test revealed no significant intergroup differences for completely occluded, partially occluded, or non- occluded tubules. The p-value for partially occluded tubules ($p = 0.071$) approached significance, suggesting potential trends that may become significant with larger sample sizes.[58]

Pairwise Mann–Whitney U tests showed selective significance for total tubule counts rather than occlusion patterns, indicating that observed differences may be attributable to specimen variability rather than true differences in formulation efficacy.[58] The use of a customized powered brushing protocol minimized procedural variation; however, in vitro conditions cannot fully replicate intraoral factors such as salivary flow, pellicle formation, and masticatory forces, which may influence occlusion stability in vivo. [66,67] The findings of the present study are broadly consistent with a growing body of in vitro and clinical literature evaluating the effectiveness of herbal desensitizing agents in achieving dentinal tubule occlusion.[33,34,47] Several previous investigations have demonstrated that herbal formulations are capable of producing meaningful tubule occlusion, although the magnitude and consistency of this effect vary depending on formulation complexity, active ingredients, and evaluation methodology.[35,48] Midha et al. (2021), in a scanning electron microscopic evaluation of desensitizing dentifrices containing potassium nitrate, calcium sodium phosphosilicate (NovaMin), strontium chloride, and a herbal formulation, reported that the herbal dentifrice achieved a moderate degree of tubule occlusion, ranking below NovaMin but above potassium nitrate alone.[48] Their findings emphasized that while NovaMin demonstrated superior occlusion due to its bioactive glass mediated remineralization mechanism, herbal formulations nonetheless produced substantial tubule blocking through surface deposition and precipitate formation.[48] The present study corroborates these observations by demonstrating that multiple herbal formulations, when tested under standardized conditions, achieve comparable levels of tubule occlusion without statistically significant intergroup differences. [21,38]

Similarly, Naghsh et al. (2024) compared a propolis-based herbal toothpaste with 5% sodium fluoride varnish and reported that the herbal formulation significantly reduced the number of open dentinal tubules when compared to both varnish-treated and untreated control groups.[32] Their findings are particularly relevant, as they challenge the conventional assumption that professional fluoride-based treatments are inherently superior to herbal formulations. The present study extends this evidence by demonstrating that not only propolis-based formulations but also herbal dentifrices containing oxalate-rich plant extracts such as spinach and rhubarb can achieve comparable tubule occlusion under in vitro conditions. [26,46] Additional studies have further reinforced the occluding potential of herbal agents. Kaur et al. (2020) evaluated an herbal desensitizing toothpaste using SEM analysis and reported complete or partial occlusion of dentinal tubules in all treated samples compared to controls, concluding that herbal dentifrices are effective in managing dentin hypersensitivity at the structural level.[47] Kar et al. (2019), although primarily a clinical study, demonstrated that herbal toothpastes significantly reduced dentin hypersensitivity scores over a four-week period, albeit to a lesser extent than 8% arginine formulations.[49] These clinical findings align with the present in vitro observations, suggesting that structural tubule occlusion achieved by herbal formulations translates into clinically perceptible sensitivity relief. [22,49] The literature also highlights considerable heterogeneity in outcomes across studies evaluating herbal desensitizing agents.[50] Bologa et al. (2023) demonstrated that different desensitizing toothpastes produced variable degrees of tubule occlusion and mineral deposition, emphasizing that formulation characteristics such as particle size, pH, and carrier matrix significantly influence outcomes.[50] This observation aligns with the present study's finding of substantial variability in standard deviations across all groups, reinforcing the notion that dentinal tubule occlusion is influenced not only by active ingredients but also by formulation and substrate characteristics.[41,62] Importantly, systematic analyses of desensitizing dentifrices have noted a steady increase in the number and diversity of herbal formulations evaluated over the past decade.[20,54] A large-scale review of randomized controlled trials assessing dentin hypersensitivity treatments reported that herbal and naturally derived agents have demonstrated progressively improving efficacy, narrowing the performance gap between conventional chemical agents and botanical alternatives. [19,20] The present study contributes to this evolving evidence base by demonstrating that diverse herbal formulations—despite differences in composition—exhibit comparable tubule occlusion capacity when assessed using uniform methodology. [35,43]

Taken together, the existing literature and the findings of the present study support the conclusion that herbal desensitizing toothpastes should not be viewed as inferior substitutes for conventional agents but rather as mechanistically distinct alternatives capable of achieving effective dentinal tubule occlusion. While bioactive glass formulations may offer superior remineralization potential, herbal dentifrices provide meaningful occlusion through precipitation and surface deposition mechanisms, validating their role in contemporary dentin hypersensitivity management. The study employed 13 specimens per group based on standard sample size calculations. However, the observed standard deviations were higher than anticipated, indicating substantial biological variability. [12,63] While the sample size was sufficient for primary comparisons, future studies with larger sample sizes may help detect subtle differences between formulations if present. [70]

Limitations of the Study

The limitations of the present investigation include:

- Absence of acid challenge testing [51]
- Use of a single SEM magnification and single field per specimen [35]
- Lack of quantitative depth analysis of occlusion deposits [42]

CONCLUSION

Within the limitations of this study following conclusions can be drawn from the study:

1.No statistically significant differences were observed among the formulations with respect to completely occluded, partially occluded, or non-occluded dentinal tubules, despite numerical variations in mean values.

2.It can be concluded that all four desensitizing toothpastes evaluated demonstrated comparable effectiveness in occluding dentinal tubules.

3.These findings indicate that herbal desensitizing toothpastes, irrespective of differences in botanical composition, are capable of achieving effective dentinal tubule occlusion through established physical and biochemical mechanisms.

4.The study provides mechanistic support for the use of herbal formulations as viable alternatives to conventional desensitizing agents in the management of dentin hypersensitivity.

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