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EVALUATION OF THE EFFICACY OF ASHWAGANDHA AND GINGER PASTE IN COMPARISON TO COMMERCIALLY FLUORIDE-BASED TOOTHPASTE ON DENTINAL TUBULES OCCLUSION AFTER ACID CHALLENGE USING SCANNING ELECTRON MICROSCOPE: IN VITRO STUDY

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ABSTRACT

Background: Dentin hypersensitivity is a common dental problem, so there is a growing interest in herbal-based products for treating oral diseases. This study assessed the efficacy of novel herbal-based toothpaste in reducing dentin hypersensitivity compared to commercially available toothpaste.

Materials and Methods: Thirty dentin discs with (1.0± 0.1mm) thickness were prepared and divided into two groups (n=15): Group 1 samples were not subjected to acid challenge. Group 2: samples were subjected to an acid challenge. Each group (n=5) was subdivided into three subgroups according to the toothpaste used: Subgroup (A): dentin discs subjected to 0.5% Ashwagandha paste. Subgroup (G): dentin discs subjected to 0.5% Ginger paste. Subgroup (F): dentin discs subjected to Fluoride-based toothpaste (+ve control). Samples were assessed for the percentage of opened dentinal tubules per surface area using a Scanning Electron Microscope (SEM) and Image J Analysis at baseline, after etching using 40% citric acid for 30 seconds, and after 14 days of the treatment regimen.

Results: The highest percentage of opened dentinal tubules per surface area was recorded in the subgroup G1 (7.23±2.45), followed by F1 (6.47±3.15), and then A1 (5.9±1.7) with no statistically significant difference (p = 0.685). However, the highest percentage of opened dentinal tubules per surface area was recorded in the subgroup A2 (2.59±0.96), followed by G2 (2.34±0.81), then F2 (0.39±0.18) with a statistically significant difference (p=0.003). Group 2 showed statistically significantly higher values regarding the percentage of opened dentinal tubules per surface area than Group 1.

Conclusion: All the toothpastes tested successfully treated dentin hypersensitivity concerning decreased opened dentinal tubules per surface area. Ginger and Ashwagandha were efficient as natural products that could successfully replace commercial products in managing dentin hypersensitivity.

KEYWORDS: Dentin Hypersensitivity, Ashwagandha, Ginger, natural products, fluoride toothpaste

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INTRODUCTION

Dentin hypersensitivity (DH) is one of the clinical conditions in daily dental practice [1]. It is characterized by sharp, short pain from exposed dentin when subjected to any stimulus, as chemical, osmotic, thermal, tactile or evaporative stimulus[2]. Such stimulus approaches the exposed dentin, causing movement of the dentinal fluid, stimulating the processes of the odontoblastic cells, and causing pain [3,4].

DH can be managed by disrupting nerve impulse transmission using potassium salts. These salts diffuse through the dentinal tubules, depolarizing the nerve cells that become unresponsive to excitatory stimuli [5]. The low-power laser is one of the treatment options that can interfere with the cell membrane's polarity, blocking the transmission of painful stimuli [6].

The second strategy in managing DH is to occlude the opened dentinal tubules of the exposed dentin surface using arginine, oxalates, strontium, and glutaraldehyde. All these elements can occlude the entrance of the opened dentinal tubules and avoid the tubular fluid movement [7]. The remineralizing agents as fluoride can precipitate fine particles that are able to occlude the entrance of the opened dentinal tubules. In addition, the high-power laser can melt the crystals of dentin surface by heat transmission, narrowing the diameter of the opened tubules [8].

Natural products are used more frequently in medicine and, subsequently, dentistry. Several studies have shown their efficacy in caries prevention, DH management, and gingival inflammation management [9].

Therefore, the current study aimed to compare the effectiveness of two natural-based toothpastes with fluoride-based toothpastes in treating dentin hypersensitivity. The null hypothesis was that no difference exists between Ashwagandha and Ginger-based toothpaste and fluoride-based toothpaste in occlusion of dentinal tubules and treatment of dentin hypersensitivity.

MATERIAL AND METHODS

Materials

Materials used in this study are:

a. 0.5% Ashwagandha paste (powder obtained from Imtenan, Egypt)

b. 0.5% Ginger paste (powder obtained from Imtenan, Egypt)

c. Sensodyne rapid action toothpaste for sensitive teeth (Contains Sodium Fluoride 0.0721% w/w and Stannous Fluoride 0.454% w/w, (1450ppm Fluoride).

Study Design

Thirty extracted maxillary and mandibular molar teeth were collected. Thirty dentin discs of (1.0± 0.1mm) thickness were prepared from the extracted teeth. Dentin discs (n=30) were randomly divided into two groups: Group 1 samples were not subjected to acid challenge (n=15). Group 2: samples were subjected to acid challenge (n=15). Each group was subdivided into three subgroups according to the toothpaste used: Subgroup A: dentin discs subjected to 0.5% Ashwagandha paste (n=5). Subgroup G: dentin discs subjected to 0.5% Ginger paste (n=5). Subgroup F: dentin discs subjected to Fluoride-based toothpaste (+ve control) (n=5). Samples were evaluated for the percentage of opened dentinal tubules using a Scanning Electron Microscope (SEM) and Image J Analysis at baseline after etching with 10% citric acid for 120 seconds and after 14 days of the treatment regimen.

Sample size calculation

The effect of different treatments on the occlusion of the dentinal tubules in 2 groups (with/without acid challenge) was evaluated using an

independent t-test or an equivalent non-parametric test to compare both groups. In contrast, an ANOVA test or an equivalent non-parametric test will be used to compare subgroups. According to Ashraf and Aidaros (2021) (10), the percentage of occluded dentinal tubules varied from (88.2±1.30) in the no acid challenge group compared to (82±2.35) in the acid challenge group. Using G power statistical power Analysis program (version 3.1.9.4) for sample size determination (11). A total sample size (n=40; equally divided into 20 in each group, 5 in each subgroup) will be sufficient to detect a large effect size (d) =0.92, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test.

Research Ethical Approval

Ethical approval was gained from the Institutional Review Board Organization (IORG0010866), Faculty of Oral & Dental Medicine, Ahram Canadian University, research number (IRB00012891#83).

Elemental analysis of Ashwagandha and Ginger powders using Inductively coupled plasma optical emission spectrometry (ICP-OES)

The levels of Ca and P were determined using an optical emission spectrometer, ICP-OES 5800 (Spectro Analytical Instruments, Agilent, USA). The wavelengths for Ca and P were 396.847 and 177.434 nm, respectively. Each determination was performed in triplicate, and the analytical procedure was validated.

Preparation of the experimental toothpaste

Development of Extract-loaded liposomal system

All pastes were prepared by Nanogate (Nanogate Company-Egypt). Tween 80 extra pure was supplied from Loba-Chemie, India, Methanol for liquid chromatography was supplied from Millipore Merck, lecithin was supplied Neogen, and sodium deoxycholate monohydrate was supplied from MP. The plant extract (Ashwagandha or Ginger) is derived from plant leaves grown in Egypt and is commercially available in (Imtenan, Egypt). To obtain an extract, 10 g of the particular leaves were ground and added to 100 mL of distilled water. After that, the solution was boiled at 80°C for 12h and filtered twice through a paper filter (figure 1).

Lecithin, Tween 80, and Deoxycholate were dissolved in methanol. The methanol was evaporated by rotary evaporation (D-Lab with Water bath, USA) under reduced pressure above the lipid transition temperature to form a lipid film on the wall of the flask. The film was hydrated at 45° C using a saline phosphate buffer solution (pH 7.4) (100ml) containing the extract by rotation at 150 rpm (12, 13,14,15). The transferosomes formed were allowed to swell for two hours at room temperature.

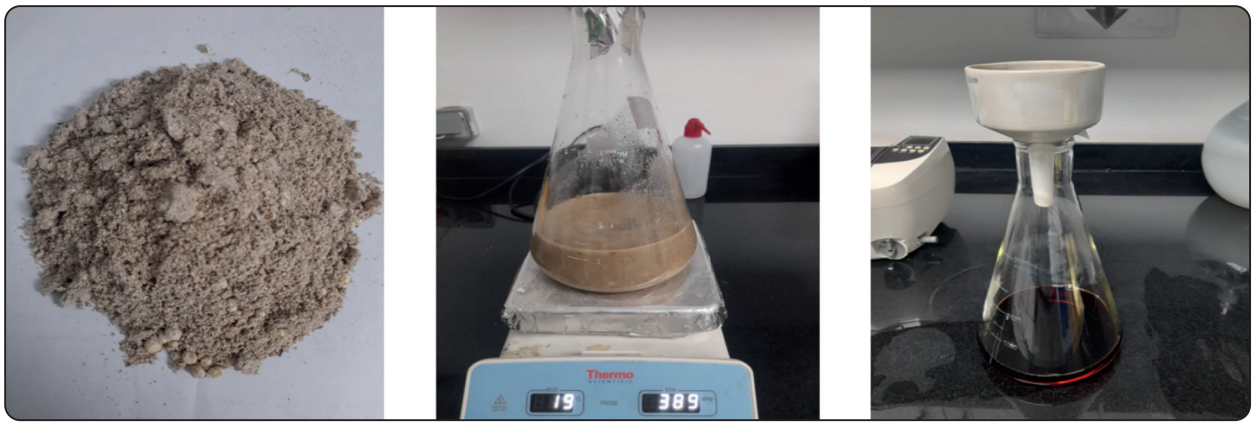

Fig. (1): Illustration of the extraction process

The resulting vesicles were sonicated for 30 min in a probe sonicator to decrease their size uniformly. The prepared transferosomes were stored in an airtight container that was well closed for further study. The composition of these formulations is shown in Table 1.

TABLE (1) Formulation code and variable used in the preparation of liposomal extract:

Formula	Lecithin	Tween 80	Deoxycholate	Extract	Cholesterol
F	180	12	8	40	10

Paste preparation

0.5 % w/v Liposomal extract paste was prepared using carbopol 940 as a gelling agent. Briefly, 0.5 gm of carbopol 940 was dissolved in 50 ml aqueous liposomal extract (AKA, Ginger or Ashwagandha) 0.5% via gentle stirring (450 rpm). The pH was adjusted to 6.5:7 by adding Triethanol amine TEA (Loba-Chemie, India). Finally, the 0.5% Liposomal extract paste was obtained.

Morphological Examination of the prepared pastes using Transmission Electron Microscope (TEM) analysis

0.5% Ashwagandha paste and 0.5% Ginger paste were characterized using a Transmission Electron Microscope (TEM) on a JEOL JEM-2100 high-resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively. Samples for TEM were prepared by placing a droplet of colloid suspension in respective solvent on a Formvar carbon-coated, 300-mesh copper grid (Ted Pella) and allowing them to evaporate in air at ambient conditions. Size distribution and average size were determined using an image analysis software package.

Zeta potential and dynamic light scattering (DLS) measurement

The charge density and DLS were measured via a Malvern analytical zeta sizer.

Artificial saliva preparation

Artificial saliva was prepared by mixing 500 ml distilled water, 20 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, carboxymethyl cellulose, 20 ml tricalcium phosphate, and 0.05 M sodium hydroxide to maintain a pH of 6.8 [16].

Demineralizing solution preparation

The demineralizing solution was based on 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid, and 1 M potassium hydroxide (KOH) to maintain a pH of 4.4 [11]

Specimen preparation

Thirty extracted maxillary and mandibular molars were collected, cleaned, and then disinfected in a 10% chloroxylenol solution [17]. The prepared discs with a thickness of 1.0± 0.1mm were prepared by sectioning below the dentin-enamel junction perpendicular to the teeth's long axis using a low-speed diamond saw and water cooling. The discs were then wet-ground for 60 seconds by using silicon carbide polishing sheets (600–1,000 grits), and a polishing stone was used to achieve a uniformly smooth surface. To remove the polishing abrasive, the polished dentin discs were put in a jar with distilled water [12]. To simulate the sensitive tooth model with opened dentinal tubules, all dentin discs were immersed for 30 s in 40% citric acid [18] as (the etching phase), rinsed with a considerable amount of distilled water, then ultrasonicated in distilled water three times, for ten minutes each time. Dentin discs were ready for studying dentine hypersensitivity when the dentinal tubules were exposed [19].

Treatment of samples:

To mimic the oral circumstances, the discs in each subgroup were inserted in a plastic container that held 10 milliliters of artificial saliva. The treatment method and the agent employed were labeled on each container.

Group 1 (samples were not subjected to acid challenge) involved subgroups (A1, G1, and F1) receiving therapy with Ashwagandha, Ginger paste, and Sensodyne toothpaste. After applying the paste to the sample surface for two minutes using a micro brush, they were rinsed for thirty seconds with distilled water and kept at 37°C in a container with artificial saliva. For 14 days, this treatment protocol was carried out once every 12 hours. Every 24 hours, the artificial saliva was replaced (10).

For Group 2 (samples were subjected to acid challenge), the subgroups (A2, G2, and F2) were treated with the same toothpaste (Ashwagandha paste, Ginger paste, and Sensodyne toothpaste) with the same protocol as Group 1. Treated samples were submerged in 10 ml demineralizing solution in a petri dish for 30 minutes. After rinsing each sample in distilled water to remove the acid for two minutes, they were put back in containers with artificial saliva at 37°C; artificial saliva was replaced every 24 hours. This process was performed every 12 hours for 14 days (10).

Assessment of the percentage of opened dentinal tubules using Scanning Electron Microscope (SEM) and Image J Analysis

For the evaluation of opened dentinal tubules using SEM, all samples were coated with gold spattering before SEM assessment in a Quick Coater vacuum evaporator (Type SC-701; Sanyu Electron Co., Tokyo, Japan) in an attempt to avoid the build-up of electrostatic charge. Opened dentinal tubules were evaluated using a Field Emission-Scanning Electron Microscope (FE-SEM) (QUANTA FEG250) accelerated voltage at 20KeV, Holland.

The image analysis for SEM Images was processed using Image J software (version 1.53a National Institutes of Health, USA). The entire image area was automatically measured in μm^2, and then the total area of opened dentinal tubules was calculated as % of the total image area using the following equation. The image analysis steps and measurement technique can be summarized in (Figure 2).

$$\text{Open dentinal tubules\%} = \frac{\text{Total area of opened dentinal tubules } (\mu m^2)}{\text{Total image area } (\mu m^2)} \times 100$$

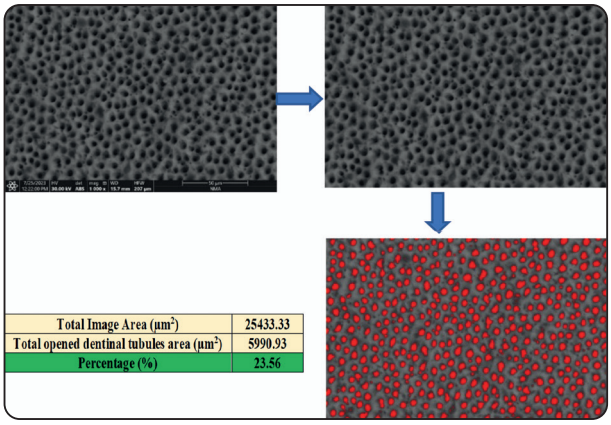

Fig. (2) Image analysis steps and measurement technique

Statistical analysis

Statistical analysis was performed using the Kruskal-Wallis test to compare the results, followed by the Mann-Whitney test for pairwise comparisons between groups and subgroups. $p \leq 0.05$ was considered statistically significant (95% significance level), and $p \leq 0.001$ was considered highly statistically significant (99% significance level). Shapiro Wilk test was used to test the normality of data. Statistical evaluation was done using the SPSS statistical package (version 25, IBM Co. USA).

RESULTS

Elemental results of Ashwagandha and Ginger powders using Inductively coupled plasma optical emission spectrometry (ICP-OES)

Elemental analysis showed the concentration of Ca and P in 100 mg of Ashwagandha and Ginger powder (table 2).

TABLE (2) Concentration of Ca and P Ashwagandha and Ginger powder:

Label	Ca (396.847 nm)	P (177.434 nm)
Blank	0.00 (ppm)	0.00 (ppm)
Standard	5.00 (ppm)	5.00 (ppm)
Ashwagandha	34251.65 (ppm)	12736.53 (ppm)
Ginger	15834.92 (ppm)	826.71 (ppm)

Morphological Examination of the prepared pastes (0.5% Ashwagandha and Ginger)

TEM analysis of the prepared 0.5% Ashwagandha paste confirmed crystalline nanoparticles of (72.2± 10) nm (Figure 3). While spheroidal shape nanoparticles of (60±10) nm for the 0.5% Ginger paste were confirmed (Figure 4).

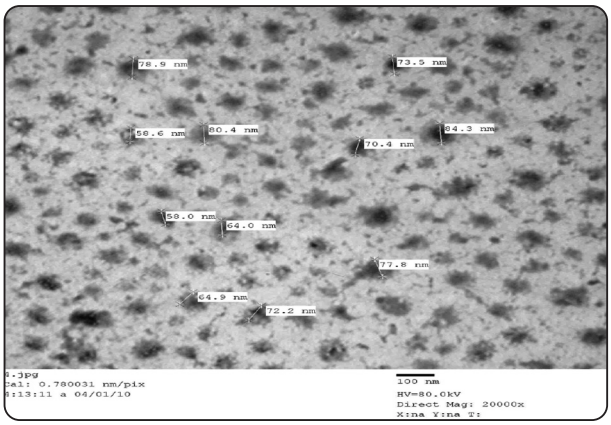

Fig. (3) TEM analysis of the prepared 0.5% Ashwagandha paste

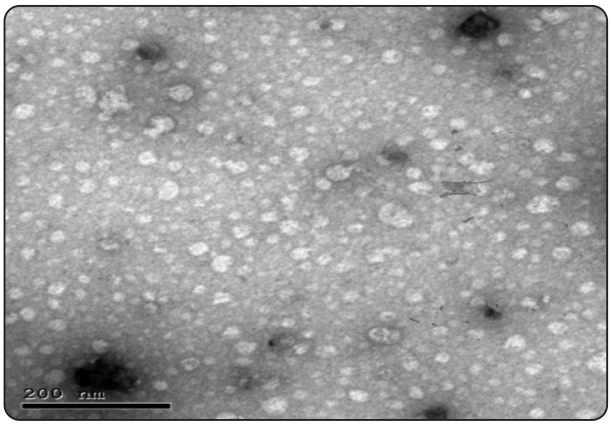

Fig. (4) TEM analysis of the prepared 0.5% Ginger paste

Zeta potential and dynamic light scattering (DLS) results

DLS revealed that the hydrodynamic size of the Ashwagandha NPs was around (241.6 nm) (Figure 5), and the zeta potential was (-27.5 mV) (Figure 6). Furthermore, DLS revealed that the hydrodynamic size of the Ginger NPs was around (242 nm) (Figure 7), and the zeta potential was (-26 mV) (Figure 8).

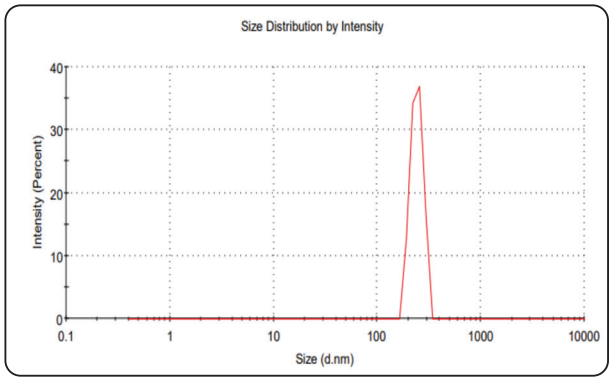

Fig. (5) DLS of Ashwagandha NPs

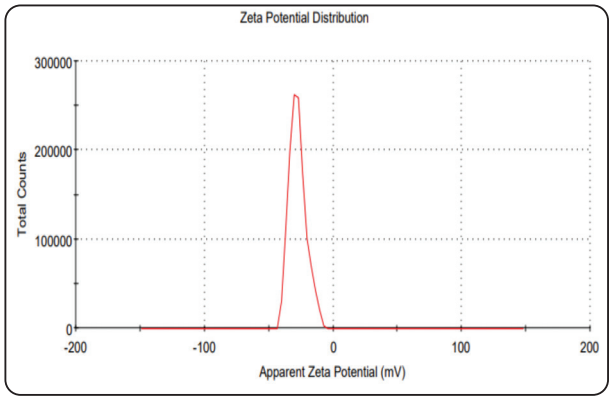

Fig. (6) Zeta Potential of Ashwagandha NPs

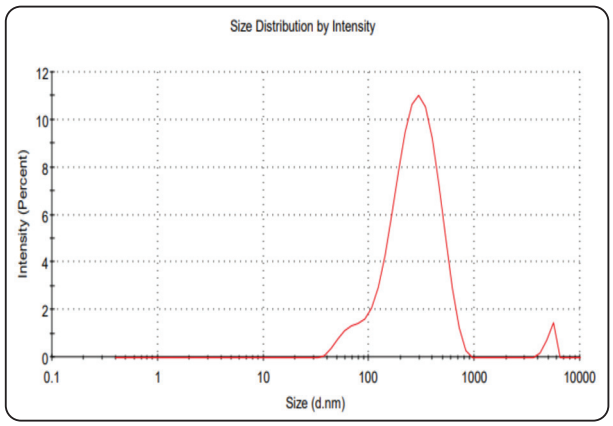

Fig. (7) DLS of Ginger NPs

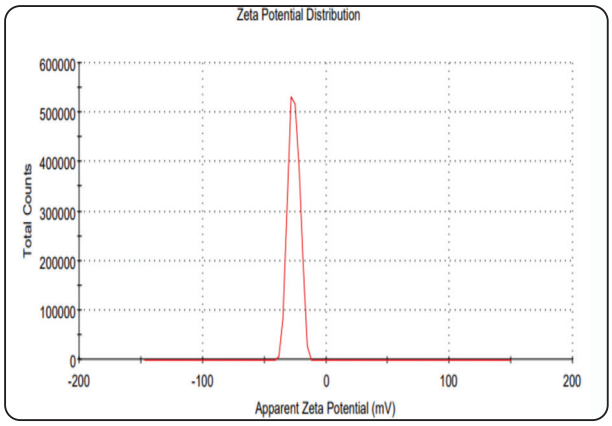

Fig. (8) Zeta Potential of Ginger Liposome NPs

The percentage of opened dentinal tubules using Scanning Electron Microscope (SEM) and Image J results

Comparison of the percentage of opened dentinal tubules using different pastes under the same major group (Intra-group comparison)

Group not subjected to acid challenge (Group 1)

For all subgroups of Group 1 (A1, G1, and F1), the highest mean percentage value of opened dentinal tubules per surface area was achieved at baseline F1 (4.53±0.46), followed by G1 (3.34±1.88) and A1 (2.57±1.07), respectively (Fig. 9a, 11a, 13a). However, the highest was achieved after etching for F1 (20.95±6.3), followed by A1 (20.71±1.3) and G1 (13.75±7.66), respectively (Fig. 9b, 11b, and 13b). After treatment with different pastes, all subgroup samples showed a decrease in the opened dentinal tubules (Fig. 9c, 11c, and 13c). The highest mean percentage value of opened dentinal tubules per surface area was recorded in the subgroup G1 (7.23±2.45), followed by F1 (6.47±3.15), and then A1 (5.9±1.7) with no statistically significant difference (p = 0.685) (table 3) (Fig. 14).

For all subgroups, according to the Mann-Whitney test for pairwise comparison between different stages, there was no significant difference in the mean of opened dentinal tubules between baseline, after-etching, and after-treatment stages.

Group subjected to acid challenge (Group 2)

For all subgroups of Group 2 (A2, G2, and F2), the highest mean percentage value of opened dentinal tubules was achieved at baseline G2 (5.19±3.22), followed by A2 (3.33±2.46) and F2 (3.08±0.95) respectively (Fig.10a,12a,14a). However, the highest was achieved after etching A2 (23.43±0.7), G2 (22.17±4.98), and F2 (19.34±6.03), respectively (Fig.10b,12b,14b). After treatment with different pastes, all subgroup samples showed a decrease in the opened dentinal tubules (Fig.10c,12c,14c). The highest mean percentage value of opened dentinal tubules per surface area was recorded in the subgroup A2 (2.59±0.96), followed by G2 (2.34±0.81), then F2 (0.39±0.18) with a statistically significant difference (p=0.003) (table 3) (Fig. 15).

For all subgroups, according to the Mann-Whitney test for pairwise comparison between different stages, there was no significant difference in the mean of opened dentinal tubules between baseline and after-treatment stages; however, there was a significant difference between the etching stage and the other two stages.

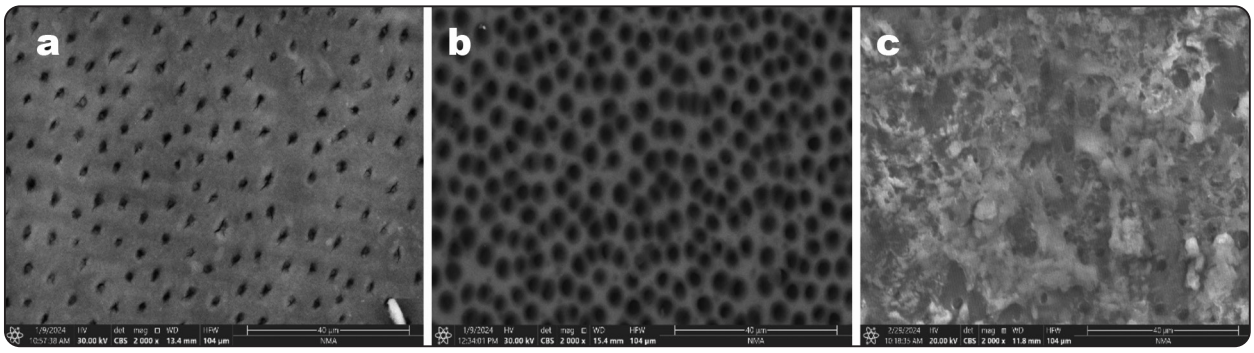

Fig. (9) SEM micrograph for dentin samples treated with 0.5% Ashwagandha group (1): (a) baseline (b) after etching (c) after treatment (under 2000x magnification)

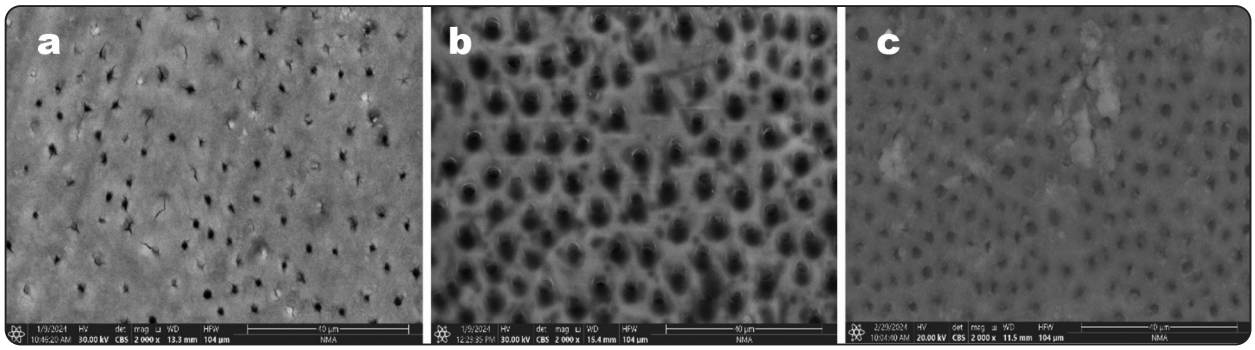

Fig. (10) SEM micrograph for dentin samples treated with 0.5% Ashwagandha group (2): (a) baseline (b) after etching (c) after treatment (under 2000x magnification)

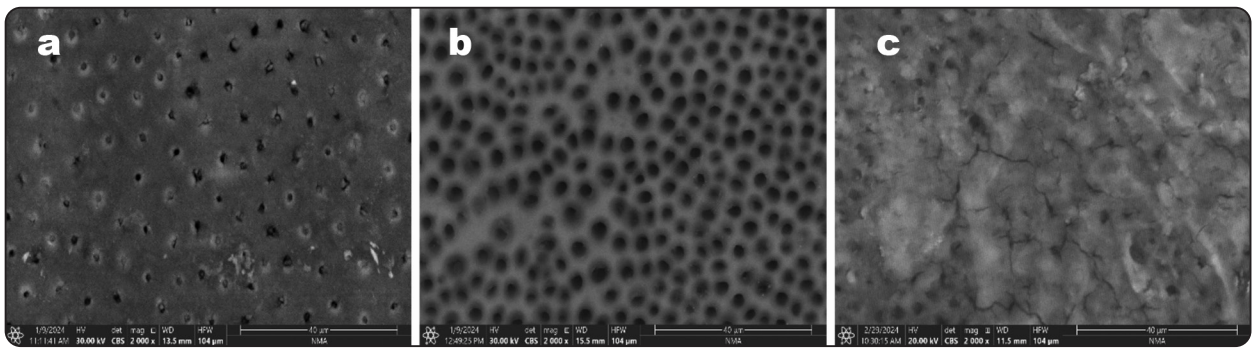

Fig. (11) SEM micrograph for dentin samples treated with 0.5% Ginger group (1): (a) baseline (b) after etching (c) after treatment (under 2000x magnification)

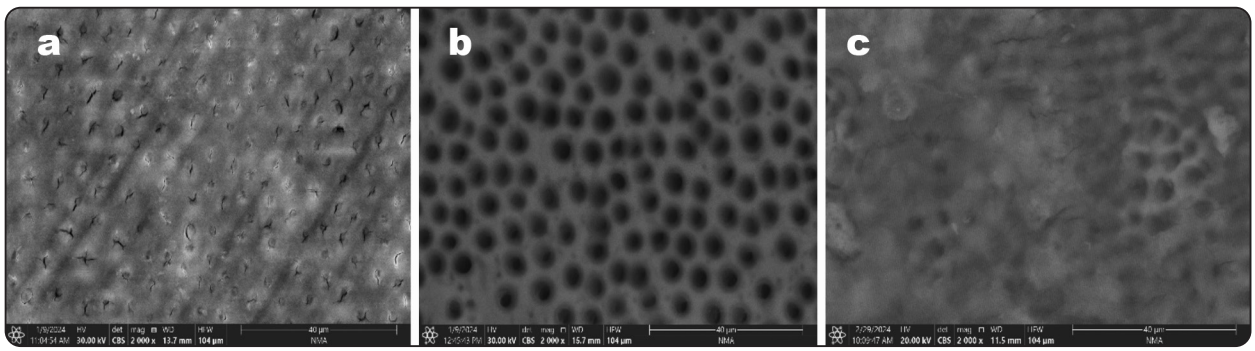

Fig. (12) SEM micrograph for dentin samples treated with 0.5% Ginger group (2): (a) baseline (b) after etching (c) after treatment (under 2000x magnification)

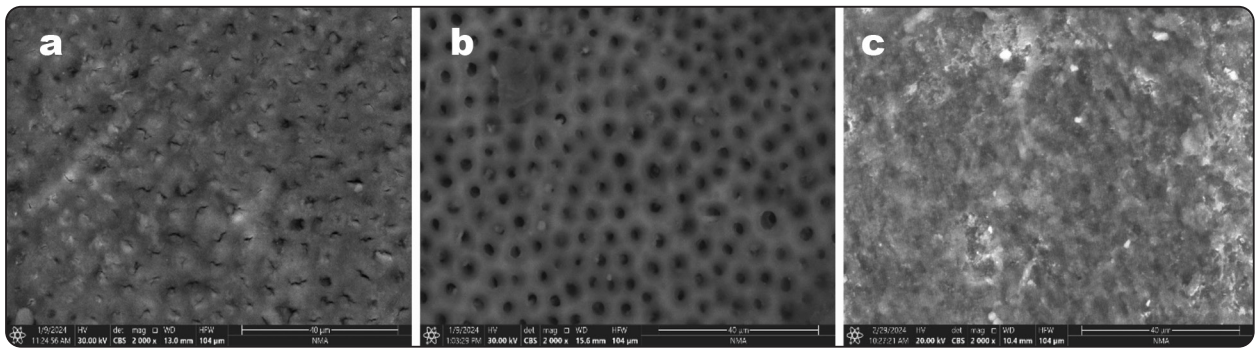

Fig (13) SEM micrograph for dentin samples treated with Fluoride group (1): (a) baseline (b) after etching (c) after treatment with Fluoride toothpaste (under 2000x magnification)

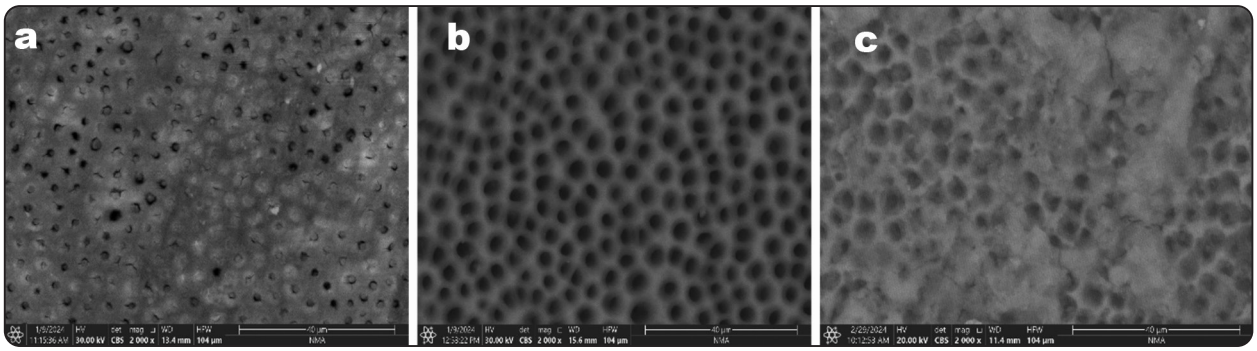

Fig. (14) SEM micrograph for dentin samples treated with Fluoride group (2): (a) baseline (b) after etching (c) after treatment with Fluoride toothpaste (under 2000x magnification)

TABLE (3) Mean ±SD and intra-group comparison of opened dentinal tubules percentage for the three stages under the major groups and subgroups studied.

Group	Subgroup	Baseline	After Acid	After Treatment	P-value*
Without pH Cycling	A1	2.57±1.07^{b}	20.71±1.3^{a}	5.9±1.7^{b}	< 0.001^{HS}
	G1	3.34±1.88^{b}	13.75±7.66^{a}	7.23±2.45^{b}	0.005^{S}
	F1	4.53±0.46^{b}	20.95±6.3^{a}	6.47±3.15^{b}	0.003^{S}
	P-value*	0.054^{NS}	0.221^{NS}	0.685^{NS}	
With pH Cycling	A2	3.33±2.46^{b}	23.43±0.7^{a}	2.59±0.96^{b}	0.003^{S}
	G2	5.19±3.22^{b}	22.17±4.98^{a}	2.34±0.81^{b}	0.001^{HS}
	F2	3.08±0.95^{b}	19.34±6.03^{a}	0.39±0.18^{b}	< 0.001^{HS}
	P-value*	0.166^{NS}	0.293^{NS}	0.003^{S}	

-* Overall P-value for Intra-group comparison between the three stages of treatment (Kruskal-Wallis test).

-**Overall P-value for Intra-group comparison between the three subgroups (Kruskal-Wallis test).

- Small letters for intra-group comparison between the three stages of treatment (Mann-Whitney test), and there is no significant difference between the means that shared at least one superscript letter at a significant level of 0.05.

- S= Statistically significant at P ≤ 0.05 - NS= Non-significant P < 0.05

- HS= Highly significant at P ≤ 0.001

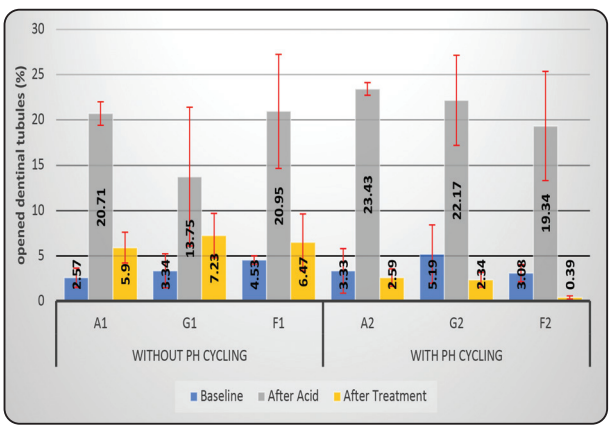

Fig. (7): DLS of Ginger NPs

Comparison of the percentage of the opened dentinal tubules per surface area for subgroups using the same paste with or without acid challenge (Inter-group comparison) between etching and treatment stage

Ashwagandha subgroup: The percentage of opened dentinal tubules per surface area showed a statistically significantly higher value with the acid challenge group (20.84±0.65) in comparison to without acid challenge (14.81±1.64) (p>.00001) (table 3) (Fig. 16).

Ginger subgroup: The percentage of opened dentinal tubules per surface area was statistically significantly higher with the acid challenge group (19.83±5.2) than without acid challenge (6.53±9.4) (p =.020371) (table 3) (Fig. 16).

Fluoride subgroup: The percentage of opened dentinal tubules per surface area was higher in the acid challenge group (18.95±5.85) than without acid challenge (14.48±8.5) with no significant **difference (p = .314019)** (table 3) (Fig. 16).

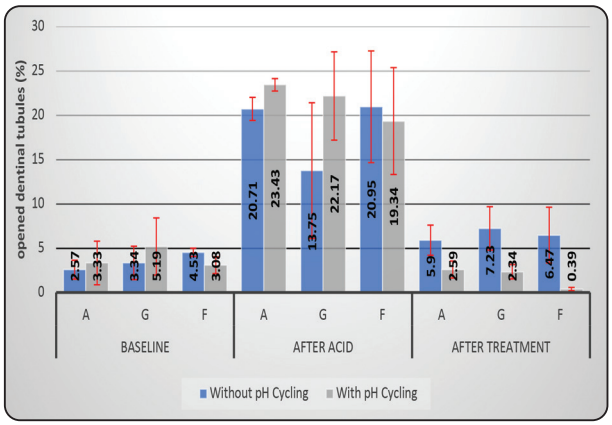

Fig. (16) A bar chart representing the mean and SD of opened dentinal tubules percentage for the three stages under the major groups and subgroups studied

DISCUSSION

Dentin hypersensitivity is a commonly presenting clinical condition in everyday practice. Dentin hypersensitivity may influence a patient's quality of life. Unfortunately, patients usually do not consider dentin hypersensitivity as an essential problem, so they do not seek treatment. The etiology still needs to be clarified, and different theories have been suggested. The most accepted theory is the hydrodynamic theory. This theory postulates that in response to various stimuli, such as chemical, tactile and thermal stimuli, the fluid flow is altered inside the dentinal tubules of the exposed dentin, thus exciting the A-δ fibers consequently, the short and acute pain characterized as dentin hypersensitivity is triggered [20].

Nowadays, herbal products are used more in medicine and, subsequently, in dentistry. Many studies have shown the effect of herbal products in managing DH. Therefore, this study evaluated the efficacy of some natural products (Ginger and Ashwagandha) versus synthetic products (fluoride-containing toothpaste). Ashwagandha and Ginger have widely recognized medicinal properties as potent anti-inflammatory and antioxidant agents [21].

Additionally, they serve as valuable sources of essential minerals, amino acids, and phenolics. The elemental analysis of Ashwagandha and Ginger powders using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) showed a high concentration of calcium (Ca) and phosphorus (P), as seen in (table 2). These findings align with the scanning electron microscopy imaging results in this study, which confirmed the ability of these herbal extracts to remineralize dentin surface and reduce dentin hypersensitivity.

Nanoparticles (NPs) have enhanced properties including increased surface-volume ratio and higher bioactivity than regular particles. Therefore, it is assumed that nanoforms may have a more significant impact on managing DH [22].

Dynamic Light Scattering (DLS) is used to gather knowledge of the nanoparticles concerning their hydrodynamic diameter. In contrast, Zeta Potential determines the surface charge variation and colloidal stability depending on various pH ranges from 2 to 10 [23]. Ashwagandha nanoparticles showed dynamic light scattering (241.6 nm) larger than Ginger nanoparticles, which is (242 nm). These results explain the larger size of the Ashwagandha nanoparticles (72.2±10 nm) compared to the (60±10 nm) of the Ginger nanoparticles. On the other hand, Ashwagandha nanoparticles showed a lower surface charge (-27.5 mV) compared to Ginger nanoparticles (-26 mV).

Etching all samples of both groups (Groups 1 and 2) using 40% citric acid for 30 seconds

opened the dentinal tubules, simulating the sensitive tooth model, which explained the increase in the mean percentage values of opened dentinal tubules per surface area after etching compared to the baseline [8].

For those tested groups (A1, G1, F1) not subjected to acid challenge, (A1) samples receiving Ashwagandha paste showed the lowest mean percentage values of opened dentinal tubules (5.9±1.7) compared to those treated with either Sensodyne toothpaste (F1) (6.47±3.15), Ginger paste (G1) (7.23±2.45). These findings may be attributed to the high mineral content of this herbal extract (table 2), which contains the highest calcium and phosphate ions are able to create a calcium rich layer on the dentin surface, that will seal the opened dentinal tubules and physically block the sensitivity process. This is in agreement with [21, 24]. Ashwagandha and Ginger have both been individually evaluated for their remineralizing effects. Farook et al. [15] evaluated herbal materials in their study for remineralizing effects. They concluded that there was a significant decrease in the number of opened dentinal tubules after treatment with these herbal products. This is in agreement with the results of the present study, where a reduction in the number of opened dentinal tubules was noticed.

For other tested groups (A2, G2, F2) subjected to acid challenge, samples treated with Sensodyne toothpaste (F2) revealed a significant reduction in the mean percentage values of opened dentinal tubules (0.39±0.18) compared to those treated with either Ashwagandha paste (A2) (2.59±0.96) or Ginger paste (G2) (2.34±0.81). The key ingredients of this toothpaste are 0.454% stannous fluoride and inactive ingredient polymer, which will be activated when subjected to acidic attack and increase the absorption rate of phosphate and calcium from saliva to support accelerated dentinal tubule occlusion[24]. Sensodyne toothpaste is revealed as a fluoride phosphate complex that can combine with calcium from saliva and the surrounding dental structures, forming new minerals; which can relief pain caused by dentin sensitivity [25]. It is called acidified bioactive complex comprising organic compounds, salts, and compounds associated with silicon and phosphate enhancing the creation of fluoridated hydroxyapatite. It forms a silicate layer that is more stable and resistant to acidic attack. It is formed deep in enamel and opened dentinal tubules. This ionic alteration decreases hydroxyapatite solubility, with consequently decrease in dentinal fluid flow, thus reducing hypersensitivity [26].

The tested subgroup (G2) samples showed more stability in the acidic media than the tested subgroup (A2) samples. This could be due to the lower Zeta Potential of Ashwagandha nanoparticles (-27.5 mV) than Ginger nanoparticles (-26 mV). Zeta potential has an important role in the behavior of nanoparticles, particularly in terms of their stability and interaction with other particles. As the results of Ginger nanoparticles showed, a high zeta potential indicates strong repulsion, which generally leads to better stability of nanoparticles in suspension. Repulsive forces prevent particles from aggregating or clumping together [27].

On the other hand, the shape and size of the nanoparticles play an essential role in their remineralization potential. Smaller nanoparticles generally have a higher surface area-to-volume ratio, allowing for more interaction with dentin surfaces. Smaller nanoparticles can penetrate dentinal tubules deeper, facilitating more comprehensive remineralization. The shape of nanoparticles affects their behavior and interaction with dentine. For instance, spherical nanoparticles distribute more evenly, leading to uniform remineralization. The specific shape can also affect how the nanoparticles aggregate, impacting the overall effectiveness and stability of the remineralization process. This explains the stabilization of the Ginger nanoparticles of spheroidal shape and size of (60±10 nm) compared

to the crystalline shape Ashwagandha-nanoparticles of the size of (72.2±10 nm) (28).

According to the analysis of the results of the current study, samples of (Group 1) receiving treatment without being subjected to acid challenge showed a significant decrease in the mean percentage values number of opened dentinal tubules per surface area compared to those of (Group 2) subjected to acid challenge. This finding is in accordance with [29], who found that acidic pH below 5 can cause disruption and the smear layer removal which covers the dentin surface and obturates the dentinal tubules, thereby increasing dentinal sensitivity.

CONCLUSION

All the tested toothpaste successfully treated dentin hypersensitivity, with decreased opened dentinal tubules per surface area. Ashwagandha and Ginger were efficient natural products that could successfully replace commercial products in managing dentin hypersensitivity.

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