Histological and CBCT Evaluation of Zamzam Water (Gel) Versus Topical Fluoride in Remineralization Potential of Induced Enamel Caries (In *Vitro* Study)

Original Article

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ABSTRACT

Introduction: The preservation of the integrity of tooth structure via the use of remineralizing natural products became the new target of modern dentistry.

Aim of the Work: A new formulation of Zamzam water (gel form) was assessed in remineralizing artificially induced caries compared to fluoride gel.

Materials and Methods: One hundred and ten premolars were randomly allocated into five equal groups (22 teeth/group). Artificial carious lesions were created on all teeth except group I of sound teeth. The remaining teeth were randomly divided into: Group II (negative control): teeth were left without treatment. Group III (fluoride gel group) (positive control): teeth were treated with acidulated phosphate fluoride gel. Group IV (Zamzam 1 gel) and Group V (Zamzam 2 gel): teeth were treated with Zamzam gel of normal concentration and of high concentration, respectively. Afterward, the enamel surface was evaluated qualitatively by scanning electron microscope and quantitatively through assessing surface microhardness using Vicker's microhardness test, and via CBCT scanning of enamel density and volume.

Results: Scanning electron microscopic examination of Zamzam 1 & 2 gel groups showed a distinct enhancement in the demineralized enamel surface than the fluoride group. Also, there was a statistically significant increase ($P \leq 0.05$) in the two groups of Zamzam gel over the fluoride group regarding enamel density and enamel effective volume after remineralization, along with a statically significant increase ($P \leq 0.05$) in Zamzam gel 2 over fluoride gel group regarding microhardness.

Conclusions: Zamzam gel could be superior to fluoride gel suggesting its ability to be used not only as a treatment but also as a preventive measure avoiding the risks of fluoride toxicity.

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INTRODUCTION

Dental caries is the most numerous infectious disease representing a global health problem[1]. Caries progression is characterized by stages of demineralization followed by remineralization with resultant obvious carious lesion due to impaired balance between these periods and outspread demineralization[2]. For caries control, there are considerable strategies that aim to shift from the restorative to the preventive phase. The principal caries preventive measures include cariogenic diet limitation, elimination of dental plaque and topical application of fluoride which is considered the most widely accepted and effective means of primary caries prevention[3].

A positive impact of professionally applied topical fluoride in reversing initial carious lesions in permanent teeth has been provided by several randomized clinical researches[4–7]. This topical effect of fluoride was correlated to the modulation of tooth structure and dental plaque with the resultant increase in resistance of tooth surface against acid dissolution and caries arrest[8,9]. The most frequently used topical fluoride agents include fluoride-containing toothpastes, mouth rinses, gels, and varnishes[10]. Moreover, Water fluoridation is considered a significant and reasonable measure for the reduction of caries[11].

Zamzam water emerges from Zamzam well in Makkah city. Its role in the alleviation of illness has been reinforced. It has a potential antioxidant capacity that minimizes the impact of radiation on mice bone marrow cells suggesting the study of its use as a natural radioprotector suppressing the cytotoxic damage of radiotherapy[12]. Moreover, Zamzam water exhibited a healing power in the curing of cutaneous wounds in rats[13]. In dental practice, children consuming Zamzam water portrayed better dental status as revealed by the reduction in their mean decayed, missing, filled teeth score[14]. Also, treatment of induced erosive lesions

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of primary teeth with Zamzam water was accompanied with remarkable increase in their microhardness values attributing such effect to the distinctive properties of Zamzam water regarding its high buffering capacity, and high mineral content[15]. Zamzam water analysis revealed that it contains high levels of calcium (93±0.09 mg/L), fluoride (0.74±0.005 mg/L), sodium (210±0.08 mg/L), magnesium (42±0.09 mg/L), chloride, sulfate, nitrate, and potassium which can react with the surface of enamel enhancing the resistance to caries[16,17].

Currently, natural protective agents are preferable in usage as they have an antioxidant effect and less toxicity[18]. Consequently, Zamzam water was selected in the present study as a natural water that is rich in cariostatic minerals. Since the fluoride cariostatic mechanism is essentially topical[8], the current study developed a new methodology as Zamzam water was formulated by adding a gelling base. We believed that this gel form had the advantage of being viscous, and easy to handle. In addition, it could flow under biting pressure penetrating pits and fissures.

Determination of enamel density is considered an important key factor in evaluating the degree of enamel mineralization. It also helps in the assessment of the remineralization efficacy of various materials. Consequently, measuring enamel density using precise and non-invasive methods became a big demand, which could be fulfilled by using cone beam computed tomography (CBCT)[19]. CBCT is a marvelous 3-D imaging modality with a lower radiation dose in comparison with the multi-slice computed tomography (MSCT)[20]. It has tremendous software properties that allow measuring enamel volume, as accurate as measured by MSCT[21].

The aim of the current in *vitro* study was to evaluate the effect of Zamzam water in gel form in two concentrations on artificial induced enamel caries compared to the usage of fluoride gel via qualitative scanning electron microscope examination (SEM) together with quantitative examination of microhardness and cone beam computed tomography (CBCT) analysis. The null hypothesis of the current study was that application of topical fluoride and Zamzam gel exert the same remineralization effects on enamel.

MATERIALS AND METHODS

Study design

This study was performed as an in-*vitro* experimental study on one hundred and ten extracted sound human maxillary premolars from 16 - 22 years male patients. These teeth were cbtained from Oral Surgery and Maxillofacial Department, Faculty of Dentistry, Tanta University. The study design was performed according to with the guidelines of the Research Ethics Committee at Faculty of Dentistry, Tanta University, with the ethical Approval code (#R-OB-10-22-3). All enrolled subjects in our study were informed with full details about the aim of the study, and all subjects informed signed a consent.

Specimen selection criteria

Teeth samples were collected following specific inclusion and exclusion conditions. The inclusion criteria included sound teeth extracted for orthodontic reasons, non-carious premolar, and no evidence of white spot lesions. While the exclusion criteria were teeth with visible cracks, erosion, restoration or tooth bleaching history, developmental anomalies, and other malformations as examined by stereoscope (40× magnification, VE-S1 Binocular Stereoscope, USA).

Estimation of the sample size

The sample size and power analysis were determined utilizing Epi-Info software statistical package made by World Health organization and center for Disease Control and Prevention, Atlanta, Georgia, USA version 2002. The sample size based on the criteria (95% confidence limit and 90% power of the study) was n=21 which was increased to 22 in each group to recompense the skipped information thus enhancing the quality of the statistics.

Experimental materials

Artificial saliva (AS): It was prepared in biochemistry laboratory of Faculty of Pharmacy, Tanta University according to the composition specified by Amaechi, Higham, and Edgar (1998)[22] for artificial saliva as 3.8 ppm Mg^{2+} ($MgCl2^{\cdot 6}$ H_2O), 84.36 ppm $PO4^{3-}$ (K_2HPO_4/ KH_2PO_4) 50 ppm Ca^{2+} (calcium lactate), 0.05 ppm fluoride, 0.625 g/L KCl, 0.4 g/L carboxymethylcellulose, and 2 g/L methyl-4-hydroxybenzoate, with the pH adjusted to 7.2 using 1 M KOH.

Demineralizing gel (DG): The gel was prepared by the addition of 100 mmol/l sodium hydroxide to 100 mmol/l lactic acid to provide a definitive pH value of 4.5. Then, 6% w/v hydroxyethyl cellulose was added to the solution during strong stirring at 37°C[23]. This was performed in the biochemistry laboratories of the Faculty of Pharmacy, Tanta University.

The demineralizing solution (DS): This solution provide an acidic environment mimicking the acids media produced after a cariogenic diet. This DS consisted of 2.0 mMol / L Ca (NO3) 2·4H2O, 2.0 mMol / L KH2PO4, 75.0 mMol / L CH3COOH and the pH was decreased to 4.5 using 1 M KOH.[23].

Remineralizing materials

a. Acidulated phosphate fluoride (APF) gel: Fluoride gel, High Brightness, B.chem company, Egypt, pH 4.5. It contains 12 300 ppm F− (1.23% APF).

b. Zamzam gel: The gel was prepared from the Zamzam well water from the Al Haram in Makkah, purchased from Saudia Arabia. Preparation of Zamzam gel ntration (Zamzam 1) was according to Forghani and Devireddy[24]: Methyl cellulose gel (3% w/v) was prepared by dispersing 3 grams of the polymer in 5ml of hot Zamzam water. This dispersion was diluted with 50ml of cold Zamzam water while continuous mixing. The resulting dispersion was stored in the refrigerator for 12 hours for complete solubility. While preparation of Zamzam gel with high concentration (Zamzam 2) was done as in Zamzam 1 preparation but we used concentrated Zamzam water after boiling the normal Zamzam water until reached half of the water volume.

Study groups

One hundred and ten human maxillary premolars (n = 110) collected in this study were randomly allocated into the following five equal groups (22 teeth/group); Group I: sound teeth were kept in AS to prevent teeth dryness. Group II (demineralized group): acted as negative control, was exposed to DG. Group III (F group) acted as positive control: teeth were exposed to DG then were treated with APF. Group IV (Z1 group): teeth were exposed to DG then were treated with Zamzam gel of normal concentration. Group V (Z2 group): teeth were exposed to DG then were treated with Zamzam gel of high concentration. Then each group was randomly subdivided into two parts, each one of them containing 11 teeth and were used in this way: one part (n = 11) for SEM evaluation and the other one (n = 11) for microhardness evaluation. From each subdivided part 5 or 6 teeth were randomly selected making equal number of eleven teeth in each group to be scanned by CBCT. Selected teeth for CBCT scanning in all groups were scanned by CBCT prior to demineralization of the last four groups (baseline values) and the last 4 groups were scanned after demineralization and the last three groups were scanned after remineralization.

Specimen preparation

Teeth were collected and sterilized in an autoclave (121 °C, 15 lbs. psi)[25]. After sterilization, teeth were scaled to remove any calculus, then cleaned with pumice to remove any debris. After that, teeth were examined by stereoscope (40× magnification, VE-S1 Binocular Stereoscope, USA) for exclusion of teeth with cracks, erosion, restoration or tooth bleaching history, developmental anomalies, and other malformations. After selection, teeth were stored in AS at 37◦C during the sample preparation process. Two coats of acid-resistant nail varnish (Colorama Maybelline, Egypt) were applied to all the crown surfaces excluding a window of 5 x 4 mm in the middle third of the buccal surface[26].

Early enamel carious lesion formation & pH-cycling

Early enamel caries-like lesions, about 5 mm in length × 4 mm in width, were created at the middle third of the exposed buccal surface via application of an acidified gel system on the tooth for four days for efficient demineralization[23]. Then teeth were washed with deionized water for thirty seconds. A synthetic pellicle-like layer was allowed to form on enamel surface by keeping them in AS for 24h. After that, pH cycling was performed as reported by White[27] and Vyavhare, Sharma, and Kulkarni[28]. The pH cycling process was proposed to approximate the pH dynamics of the oral environment. This regimen consisted of subjecting specimens to the remineralizing agent according to each group. Group III were subjected to APF following the instructions of the manufacturer for 4 minutes once per week[23]. The gel was applied as a thin ribbon (5 mg) with a finger over the surface of enamel. Using gauze, gentle rubbing of the gel was done, then washing under the running deionized distilled water for thirty seconds. Whereas group IV & V were subjected to Zamzam 1, and Zamzam 2 gels respectively for 2h three times per day. Zamzam gel was applied as in group III. After that specimens were exposed to two hours of demineralization a day by immersing in a DS followed by washing with running deionized distilled water for thirty seconds then were immersed individually for the rest of the day in AS as a remineralizing solution. Group II was not exposed to any treatment and was involved in the pH cycling to ensure that the artificially induced lesion did not remineralize after pH cycling. To avoid supersaturation, both AS and DS were prepared as fresh solution every day[29]. (changed after the demineralization period). This pH cycling was carried out for 12 days[28].

Cone beam computed tomography (CBCT) analysis

The selected teeth of all groups were impeded in a radiolucent block and scanned before demineralization (baseline values), with being scanned after demineralization and after remineralization for the last three groups by Cone beam machine (KaVo OP 3-D Vision, Kavo Dental, Biberach, Germany). Teeth were scanned using fixed exposure parameters (120 Kv, 5mA and 0.125mm voxel size) with the smallest field of view (8D, 8Hcm) to improve spatial resolution. The 3-D module in the On Demand Dental software (Seoul, Korea, version 1.0, build 1.0.10.7510, ×64 Edition) was used to measure density and volume in each scan. First density was measured on 3-D modules in the middle third of the buccal region of enamel using the same axial slicing to ensure standardization with using step-5 of density to avoid measuring density in region of partial volume artifact on the periphery of enamel as shown in (Figures 1A-C) which represent scan at base, after demineralization, after remineralization respectively. The densities were measured first in all stages prior to measuring the volume of enamel. To select the proper region of interest which was lower than the lowest value of density obtained to ensure the standardization in measuring the volume of region of interest. The volume was measured on 3-D module using pick with select specific region of interest from 2700-4000 as in this case the lowest value of density was 2787 to ensure measuring the volume of the same region as shown in (Figures 1 D,E).

Scanning electron microscopic analysis

For SEM analysis, specimens were prepared by separating the crown from the root at the level of cementoenamel junction using diamond saw under watercooling. All the specimens were thoroughly dried at room temperature for 24h, and gold-plated using Blazers SCD-050 ion sputtering evaporator device. Finally, the specimens were mounted on SEM (JSM-IT200, JEOL) for examination of the morphological features of the enamel surface.

Enamel microhardness analysis

For microhardness analysis, specimens were prepared by cutting the middle third of the crown of all samples (5 mm in length × 4 mm in width) with the water-cooled diamond saw of a precision sectioning machine. All the specimens were fixed in the center of an acrylic resin plate within plastic tube, with its experimental enamel surface faced upwards. The surface of microhardness of all specimens were determined as Vickers hardness number (VHN) using digital display Vickers microhardness tester (Model HVS-50, Laizhou Huayin testing instrument Co., Ltd. China) with a Vickers diamond indenter. For microhardness measurement, the enamel surface of the specimens was placed at right angle to the indenter and a load of 100 grams was applied over it for 10 seconds. Five indentations were made on enamel surface with an equal distance between them (100 μm). The average of the five indentations was calculated for statistical analysis.

Statistical analysis

Statistical analysis was done for the assembled data using SPSS version 19 (Statistical Package for Social Studies) created by IBM, Illinois, Chicago, USA. The numerical values were presented via calculation of mean, median, and interquartile range. Because of the abnormal distribution of the data, the Kruskal-Wallis test was used to compare inter-group quantitative data with *P-value* ≤0.05 was considered a significant difference (*). Using Mann-Whitney test in case of significance to detect the difference between groups and Wilcoxon test was used to compare intra-group with *P-value* ≤0.05 was assessed as a significant difference (*).

RESULTS

Qualitative results (SEM analysis)

The morphology of enamel surface was examined before and after demineralization and then after remineralization by fluoride, Zamzam gel 1 and 2. SEM of the surface of the untreated enamel from group I displayed nearly smooth surface with few areas of enamel rod ends. After demineralization, the surface of enamel depicted rough surface with numerous exposed enamel rods that appeared as deep pits (Figures 2 a-d). Remineralized enamel surfaces with fluoride gel (group III) showed a new wide spreading crystal formation creating smaller rod ends compared to the demineralized surface. However, treated enamel surfaces with Zamzam gel 1 (group IV) showed nearly complete closure of enamel rod ends with few incompletely closed pores that led to almost smooth surfaces with shallow rod ends. Moreover, enamel surfaces treated with Zamzam gel 2 (group V) illustrated almost normal surfaces with few depressions, cracks, and pits. The gel material appeared covering some areas (Figures 3 a-f).

Statistical analysis

CBCT Analysis results

Enamel density

Comparison of baseline mean values between sound teeth, demineralized, F, Z1 and Z2 groups showed no statistically significant difference ($p > 0.05$) between them as shown in (Table 1). This revealed that all groups before the induction of artificial carious lesions were in the same range regarding enamel density.

After demineralization, comparison of the mean values of enamel density between the demineralized, F, Z1 and Z2 groups as shown in (Table 2) illustrated that there was no statistically significant difference ($p > 0.05$) between them. This indicated standardization in the demineralization process.

The demineralization of each of the last four groups was evaluated by subtracting its baseline value before demineralization from its enamel density value after demineralization. Using Wilcoxon test for intra-group comparison revealed that there was a statistically significant decrease ($p \leq 0.05$) in all groups indicating effective demineralization as shown in (Table 3).

To assess the remineralizing effect of fluoride and Zamzam gel, an inter-group comparison of the mean values of enamel density after remineralization (end-values) with enamel density of both sound and demineralized groups illustrated a statistically significant difference ($p \leq 0.05$) between them. Using Mann-Whitney test, the end-value of F group after remineralization showed a slight increase with no significant difference with demineralized group, indicating the weak effect of fluoride gel treatment. However, there was a significant increase in end-values of both Z1 and Z2 groups over F group and the demineralized group with no significant difference between the end-value of Z1 and Z2 groups and sound teeth group, indicating the superior effect of Zamzam gel (Z1 and Z2) over fluoride gel as shown in (Table 4).

Effect size of enamel volume

To confirm the standardization of enamel volume at base and after demineralization between treatment groups, an inter-group comparison of the mean values of enamel volume was performed using Kruskal-Wallis test. This revealed there was no statistically significant difference ($p > 0.05$) between the three groups. However, at remineralization there was a statistically significant difference ($p \leq 0.05$) between the three groups. With the aid of Mann-Whitney test, there was no statistically significant difference ($p > 0.05$) between Z1 and Z2 groups whereas there was a statistically significant difference ($p \leq 0.05$) between F and Z1 groups and between F and Z2 groups. This indicated the efficient remineralization of Zamzam gel (Z1 and Z2) over fluoride gel.

Also, dimensional changes in volume after demineralization were assessed by subtracting the volume of the base from the volume after demineralization. Using Wilcoxon test for intra-group comparison revealed a statistically significant difference ($p \leq 0.05$) indicating effective demineralization for all groups. Kruskal-Wallis test was used for inter-group comparison to evaluate effect enamel volume after demineralization, there was no statistically significant difference ($p > 0.05$) between all groups, indicating standardization of demineralization process.

The dimensional changes in volume after remineralization were assessed by subtracting the volume of base from the volume after remineralization. Using Wilcoxon test for intra-group comparison revealed a statistically significant increase ($p \leq 0.05$) indicating effective remineralization for all groups.

Using Kruskal-Wallis test for inter-group comparison to evaluate the effect enamel volume after remineralization, there was a statistically significant difference ($p \leq 0.05$). Mann-Whitney test was used to detect significance between groups for volume dimensional changes after remineralization which showed a statistically significant increase ($p \leq 0.05$) in Zamzam groups (Z1 and Z2), over F group with no statistically significant difference between (Z1 & Z2) groups that revealed the greatest remineralizing effect of Zamzam gel (Z1 and Z2), over fluoride gel as shown in (Table 5).

Enamel microhardness results

Using Kruskal-Wallis for comparing the mean values of microhardness for sound teeth, and for demineralized, F, Z1 and Z2 groups after pH cycling, there was a statistically significant difference ($p \leq 0.05$) between them. Mann-Whitney test revealed no statistically significant difference ($p > 0.05$) between both Z1 and Z2 groups and sound teeth group indicating the effectiveness of both concentrations of Zamzam gel in reobtaining the microhardness of sound teeth. Mann-Whitney test also revealed no statistically significant difference between F group and Z1 group. However, it showed a statistically significant decrease ($p \leq 0.05$) between F and demineralized groups compared to sound teeth group. Also, there was a statistically significant difference ($p \leq 0.05$) between the demineralized group and the remaining four groups, showing the ability of treated groups to perform effective remineralization that improved their hardness. Even more, there was a statistically significant difference ($p \leq 0.05$) between Z1 and Z2 groups in favor of Z2 group as shown in (Table 6).

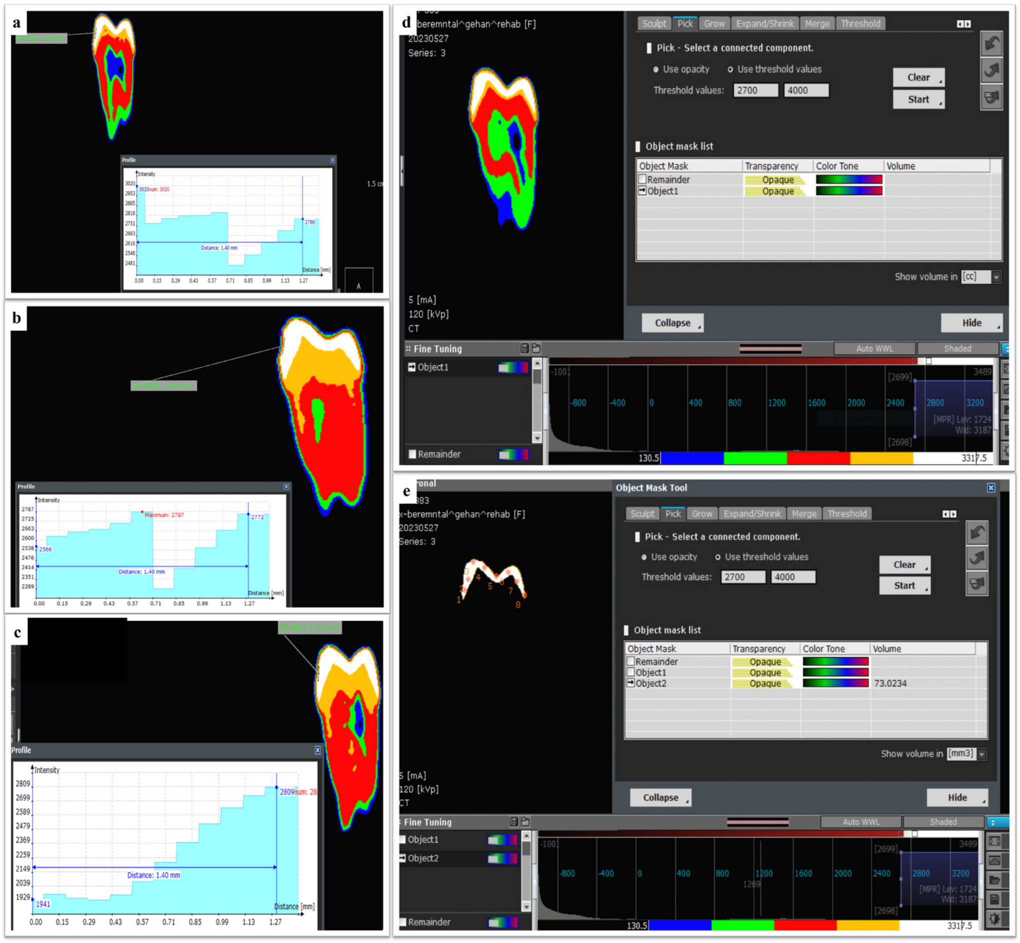

Fig. 1: Measurement of enamel density of the middle of the buccal region by CBCT at:(a) baseline value, (b) after demineralization, (c) after remineralization. (d) determining the region of interest to measure enamel volume. (e) measurement value of the volume after selecting region of interest.

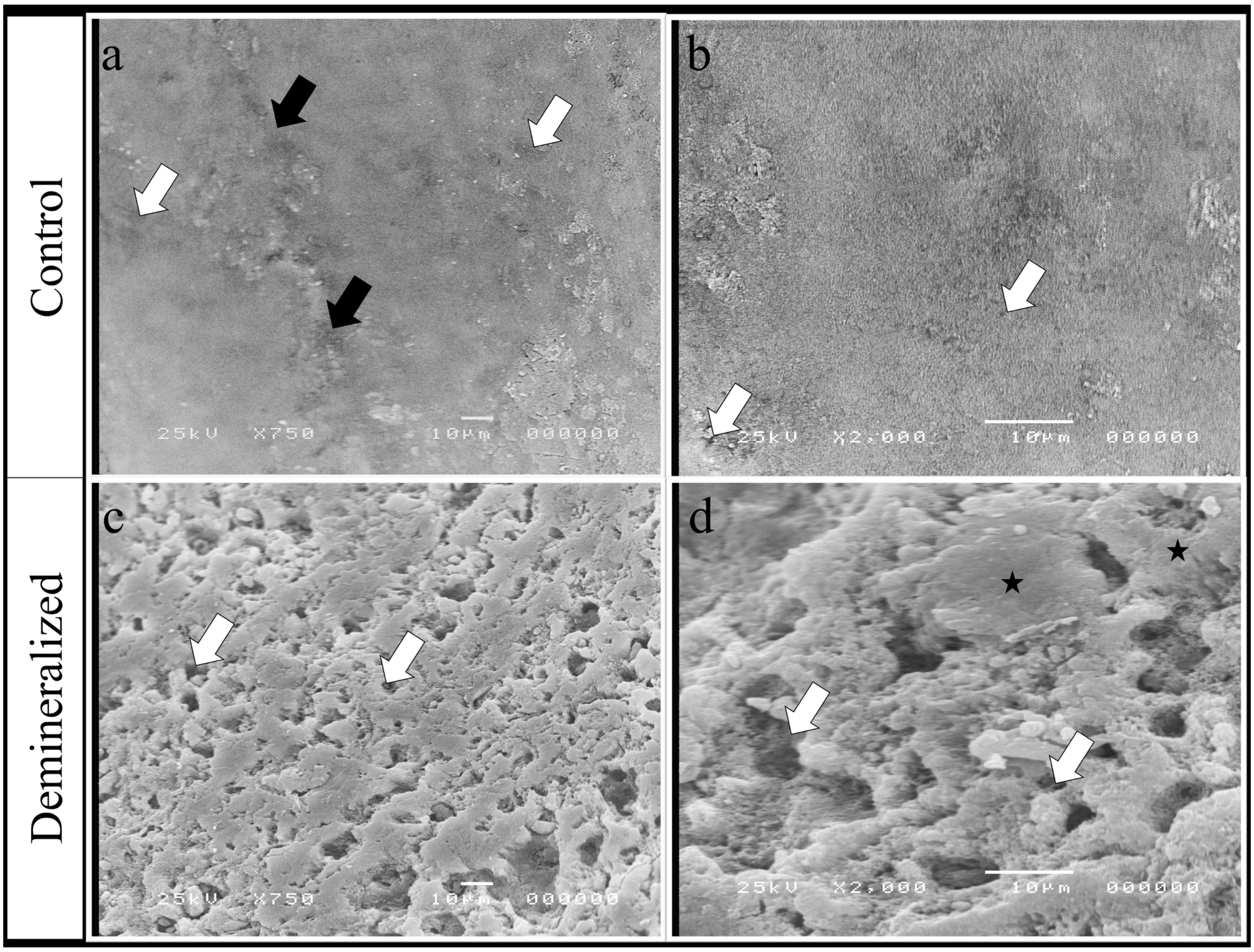

Fig. 2: SEM of the surface of enamel from group I. (a): shows a nearly smooth surface with few areas of enamel rod ends (white arrows). (b): Higher magnification showing completely covered enamel with few pits (arrows). (c): SEM of the surface of enamel after demineralization depicting a rough surface with numerous deep pits (white arrows). (d): Higher magnification showing exposed enamel rods (white arrows) along with areas of unaffected enamel surface (stars).

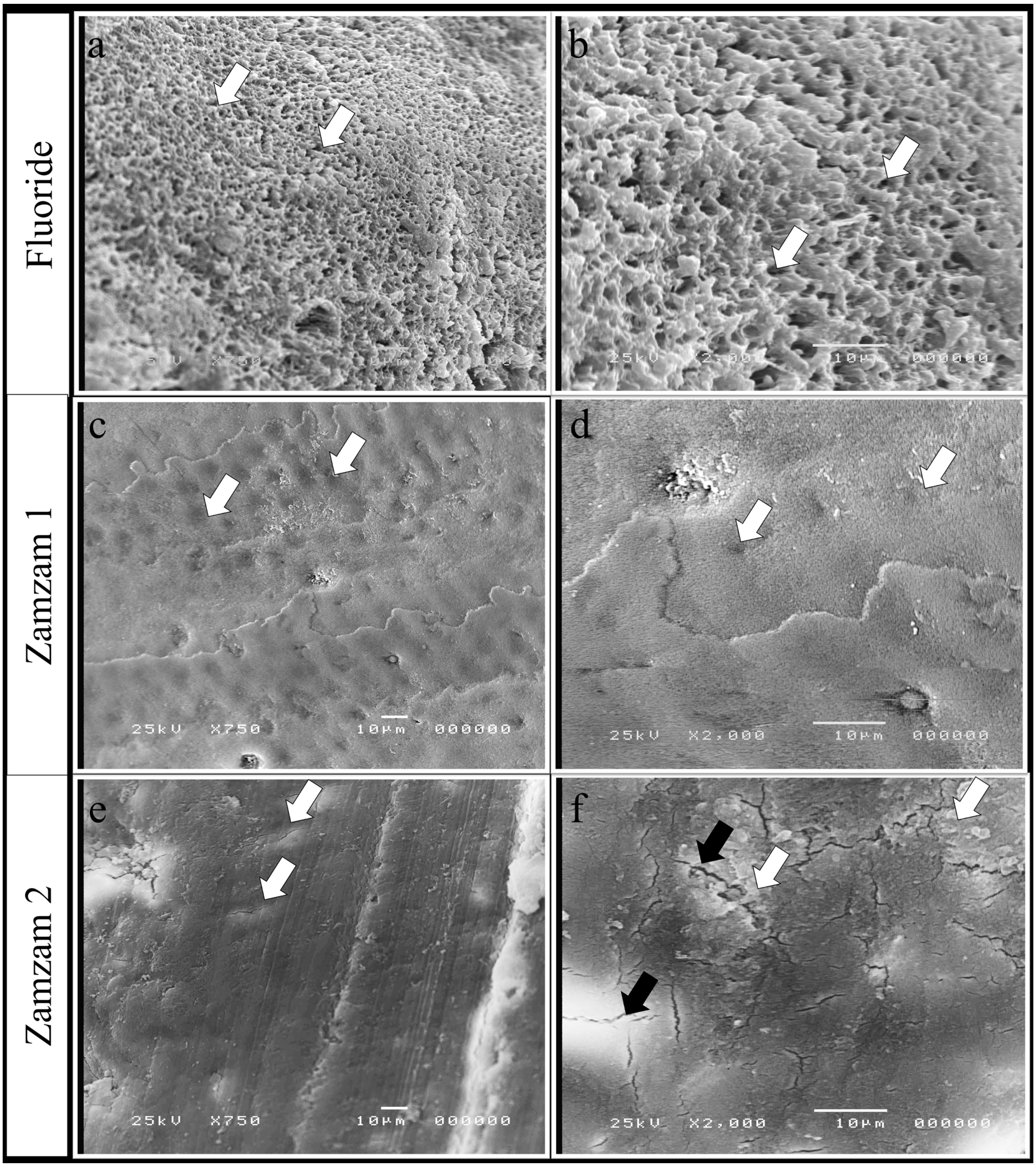

Fig. 3: SEM photographs of remineralized enamel surfaces. (a): fluoride treated enamel surface shows new wide spreading crystal formation on the surface. (b): higher magnification depicts smaller rod ends (arrows) compared to the demineralized surface. (c): Zamzam gel 1 treated surface showing nearly smooth surface with shallow rod ends (arrows). (d): higher magnification showing complete closure of the enamel rod ends and a few incompletely closed pores (white arrows). (e): Zamzam gel 2 treated surface illustrating almost normal surface with few depressions (arrows). (f): Higher magnification showing some areas covered by the gel material (white arrows) on an almost smooth surface with few cracks (black arrows).

Table 1: Inter-group comparison of baseline mean values regarding density of enamel.

Measures	Sound teeth group	Demineralized group	Fluoride group (F)	Zamzam (Z1) group	Zamzam (Z2) group
M±SD	3207±149.9	3172±87.05	3161±105.7	3222±124.0	3179±125.9
Median	3213	3211	3154	3233	3197
IQ range	3044-3342	3121-3221	3045-3266	3158-3328	3092-3277
Kruskal-Wallis test			2.574		
p			0.631		

Table 2: Inter-group comparison of mean values of enamel density after demineralization

Measures	Demineralized group	Fluoride group (F)	Zamzam (Z1) group	Zamzam (Z2) group
M±SD	2968±75.53	2938±111.1	3006±161.6	2947±117.8
Median	2986	2893	3011	2900
IQ range	2887-3021	2843-3015	2867-3131	2884-3023
Kruskal-Wallis test		1.665		
p		0.645		

Table 3: Intra-group comparison regarding enamel density before and after demineralization

Measures	Demineralized group	Fluoride group (F)	Zamzam (Z1) group	Zamzam (Z2) group
Effect size (Mean±SD)	-204.6±131.5	-223.5±42.2	-216.1±63.0	-232.2±51.7
Z	2.845	2.934	2.934	2.936
p	0.004***	0.003***	0.003***	0.003***

Test significance: p*<0.05, p**<0.01, p***<0.001.

Table 4: Inter-group comparison of mean values regarding enamel density between all groups after pH cycling

Measures	Sound teeth group	Demineralized group	Fluoride group (F)	Zamzam (Z1) group	Zamzam (Z2) group
M±SD	3207±149.9	2968±77.5	2950±114.6	3147±113.6	3206±283.9
Median	3213	2991	2934	3200	3172
IQ range	3044-3342	2888-3014	2899-3012	3022-3214	3022-3299
Kruskal-Wallis test			25.569		
p			<0.001***		
	Pairwise analysis by Mann-Whitney test				
	• Sound teeth group significantly different form demineralized group (p=0.001), Fluoride group (*p*<0.001)				
	• Demineralized group significantly different from Zamzam (Z1) (p=0.003) and Zamzam (Z2) (*p*=0.003).				
	• Fluoride group significantly different from Zamzam (Z1) (p=0.002) and Zamzam (Z2) (*p*=0.005)				

test significance: p*<0.05, p**<0.01, p***<0.001.

Table 5: Inter-group comparison of mean values of effect size of enamel volume after demineralization and remineralization

Measures	Fluoride group (F)	Zamzam (Z1) group	Zamzam (Z2) group	Kruskal-Wallis test	*p*
Volume at baseline:					
M±SD	80.8±3.8	81.9±2.6	82.6±2.6	1.280	0.217
Median	80.6	78.0	78.8		
Volume at demineralization:					
M±SD	78.2±3.8	80.4±4.1	80.0±2.1	1.478	0.151
Median	77.98	80.66	80.65		
Effect size					
M±SD	-2.6±1.1	-1.5±3.1	-2.6±1.4	0.391	0.822
Median	-2.4	-2.4	-2.1		
Z	2.934	1.956	2.936		

Measures	Fluoride group (F)	Zamzam (Z1) group	Zamzam (Z2) group	Kruskal-Wallis test	*p*
p	0.003***	0.050***	0.003***		
Volume at re-mineralization:					
M±SD	78.9±3.6	81.3±2.3	82.2.4	2.233#	0.023*
Median	82.0	80.7	81.4		
Effect size					
M±SD	-1.9±0.9	-0.6±0.5	-0.5±0.7	13.272#	0.001***
Median	-1.7	0.8	0.4		
Z	2.936	2.669	2.934		
p	0.003***	0.008***	0.003***		

#Pairwise analysis by Mann-Whitney: for both volume at remineralization and effect size:

- Fluoride group significantly different from Zamzam (Z1) ($p<0.001$) and Zamzam (Z2) ($p=0.003$)
- Zamzam (Z1) is not significant from Zamzam (Z2) ($p=0.250$)

test significance: $p^*<0.05$, $p^{**}<0.01$, $p^{***}<0.001$.

Table 6: Inter-group comparison of the mean values of microhardness

Measures	Sound Teeth group	Demineralized group	Fluoride (F) group	Zamzam (Z1) group	Zamzam (Z2) group
M±SD	423.6±41.7	325.0±30.3	384.4±35.3	400.4±43.2	438.5±21.1
Median	413.2	313.3	371.3	393.7	432.4
IQ range	38734-462.4	301.3-349.7	352.6-412.6	393.7-432.8	425.7-156.3
Kruskal-Wallis test			29.892		
p			<0.001***		

Pairwise analysis by Mann-Whitney test:

- Sound teeth significantly different form demineralized group (p=<0.001), Fluoride group (p=0.028)
- Demineralized group significantly different from Fluoride group (p=0.001), Zamzam (Z1) (p<0.001) and Zamzam (Z2) (p<0.001).
- Fluoride group significantly different from Zamzam (Z2) (p=0.002)
- Zamzam (Z1) significantly different from Zamzam (Z2) (p=0.023)

test significance: $p^*<0.05$, $p^{**}<0.01$, $p^{***}<0.001$.

DISCUSSION

Preservation of the integrity of tooth structure via the use of remineralizing agents represents an indispensable target in dental practice for early intervention of dental caries or tooth erosion[30]. Fluoride has been used as a traditional treatment that impedes the demineralization and enhances the remineralization of enamel lesions. However, there are some risk factors that have been correlated with its frequent use or overexposure that resulted in dental fluorosis development or exacerbation of existing fluoride syndrome. Hence, new methods have been investigated[31]. Therefore, the aim of the current study was to assess the potential remineralizing effect of Zamzam water (in gel form) at normal and high concentrations on the demineralized tooth surface and to compare such effect with fluoride gel application via qualitative SEM examination together with quantitative examination of microhardness and CBCT analysis.

Although much work on the possible effect of Zamzam water on enamel remineralization has been carried out, the current study developed a novel approach of Zamzam water. It was formulated by adding a gelling base in one form and by boiling Zamzam water in the other form to increase the concentration of its minerals. The gel form was believed to have the advantage of being viscous, and easy to handle. Bhongsatiern *et al*.[32] reported that fluoride in gel form had an advantage over liquid form (mouth rinse) resulting in better cariostatic effect. Being well retained on tooth structure and surrounding oral mucosa, the gel seemed to act as a reservoir for fluoride ions allowing gradual dissolution of gel into saliva and hence prolonged release of fluoride ions that can penetrate enamel surface for a greater depth compared to superficial action of the liquid form of mouth rinse. Moreover, Lee *et al*.[33] confirmed that APF gel was more efficient in remineralizing enamel surface than varnish. They correlated their findings to its high enamel fluoride concentration and to its low pH which may have etched the enamel surface increasing its roughness allowing more incorporation of fluoride into the surface. Consequently, APF gel was selected in this study to standardize the physical properties of the remineralizing agents used in the study.

After verification of the formation of the artificial caries clinically through the chalky white appearance. Teeth of group II underwent pH cycling without treatment for 12 days to confirm that they were not remineralized by AS of the pH cycling. SEM examination confirmed the presence of exposed rod ends as deep pits which agreed with the findings of Akküç *et al*.[34]. Also, CBCT analysis of enamel density after pH cycling depicted a statistically significant difference ($P \leq 0.05$) between the sound teeth group and the demineralized group.

In this study, SEM results revealed refinement of the structural features of the demineralized enamel surface after fluoride application as represented by a new wide spreading crystal formation creating smaller rod ends compared to the demineralized surface. This was in agreement with Hamze *et al*.[35] who depicted that applying fluoride gel was accompanied with outstanding reduction in the surface roughness of acid etched enamel. This effect could be mediated by the deposition of calcium fluoride onto the tooth surface that furnish a store for fluoride promoting remineralization of hard tissues[36]. Also, the incorporation of fluoride in the form of fluorapatite crystals on tooth surface rendered it more resistant to acid attack and solubility[9]. Besides its effect on tooth structure, fluoride has a topical effect on dental plaque that led to deterioration in its bacterial biofilm properties and metabolism with resultant decrease in the plaque bacterial capacity to produce considerable amounts of acids from carbohydrates and so restrain the demineralization process.

Remarkably, the evaluation of SEM micrographs revealed distinct more enhancement in the demineralized enamel surface following the application of Zamzam 1 & 2 gel treatment than the fluoride treated surface as manifested by; a visible, remarkable repair of pitted enamel surface. Moreover, Zamzam 2 group showed better surface architecture than Zamzam 1 group as it showed nearly normal enamel surface. The remineralizing potential of Zamzam water in the solution form was confirmed by Elkabbany, *et al*.[37] who reported repair of enamel surface defects of induced enamel carious lesions around orthodontic brackets after Zamzam water treatment. This may be due to enrichment of Zamzam water with minerals such as fluoride, magnesium, and calcium with their incorporation into the enamel appetite crystals increasing the resistance to acid dissolution together with the restoration of defective enamel substrate[38]. Also, reducing the surface irregularities could lead to decline in plaque accumulation on the surface enamel within the oral cavity and so could prevent caries initiation.

Noteworthy, this decrease in refinement of fluoride group could agree with Raghavan *et al*.[39] who depicted occasional roughness of enamel surface by SEM following topical APF application as revealed by areas of loss of prismatic structure that were corrected upon use of nanoparticle based APF. Also, a similar finding of roughness of enamel surface upon APF was reported in other studies accounting it to the acidic pH of APF that led to loss of minerals of surface layer with subsequent lacunae formation[40–42]. On the other hand, Zamzam water has pH of 7.9–8.0, implying that it is slightly alkaline which could explain its more improvement of surface structure[14].

Regarding our qualitative SEM examination, the quantitative analysis of the current study aimed to assess the impact of fluoride and Zamzam gel on surface microhardness, enamel density, and volume to confirm that their effect was not restricted to enamel architecture but also its mechanical properties. Concerning the microhardness, there was a significant decrease ($p \leq 0.05$) in the demineralized group compared to the sound teeth group. This could be related to the enamel mineral disintegration after application of demineralizing acid that led to dissociation of hydroxyapatite crystals with subsequent loss of high percentage of phosphate and calcium[43].

After remineralization, all the treatment groups showed a significant increase ($P \leq 0.05$) in surface microhardness compared to the demineralized group indicating a positive impact of fluoride and Zamzam gel in restoring the surface microhardness of the softened demineralized enamel. This positive effect of Zamzam 1 &2 groups was in agreement with previous studies that investigated the remineralizing effect of Zamzam water on both permanent[44] and primary dentition[15]. They suggested a potential chemical reaction between the fluoride content of Zamzam water and hydroxyapatite crystals of surface enamel augmenting the resistance to acid dissolution with the resulting increase in microhardness of artificially initiated enamel carious lesions treated with Zamzam water. Also, this increase in microhardness of Zamzam gel (Z1 and Z2) groups agreed with the study of Elkabbany, Mosleh, and Metwally[37] who referred this to the integration of minerals of Zamzam water as fluoride, calcium, and magnesium in the appetite crystals. Also, such minerals of Zamzam gel (Ca and phosphate) may form insoluble precipitates in aqueous media, reducing hydroxyapatite dissolution and increasing its resistance to caries[45].

Furthermore, the slight increase in microhardness after fluoride application was in agreement with Dionysopoulos *et al*.[46] who reported an increase in surface microhardness upon fluoride treatment after bleaching procedures. This was also in agreement with Byeon *et al*.[47] who reported support of the strength of bovine teeth structure after fluoride application as indicated by an elevation of fluoride contents inside the teeth and on its surface together with improvement of surface microhardness.

However, when comparing microhardness of F group with microhardness of sound teeth group and Z2 group there was a statically significant decrease ($P \leq 0.05$) of F group compared to sound teeth and Z2 gel groups which indicated weak effect of the fluoride gel. This coincided with Hoobi[48] who reported that the release of calcium ions into the etching solution was lesser in Zamzam water treated teeth than that released from fluoride treated group, suggesting an enhancing effect of Zamzam water than fluoride.

CBCT is considered a non-invasive 3-D image modality as being the only method, that could be used clinically in human to evaluate effect of remineralizing agents[19]. In the current study, CBCT analysis was done to assess enamel mineral density and volume. Considering the intergroup significance, the three treatment groups showed no significant difference ($p > 0.05$) in enamel density and volume with both sound teeth and demineralized groups before demineralization and between demineralized group after demineralization, ensuring optimal baseline values for subsequent intergroup comparison after remineralization.

After remineralization either with fluoride or Zamzam gel (Z1 and Z2 groups), CBCT revealed an increase in both enamel volume and density with a statistically significant increase ($p \leq 0.05$) in effect enamel volume after remineralization for all treated groups. This could be attributed to deposition of calcium fluoride like precipitates on surface enamel after topical application of APF as revealed by Raghavan *et al*.[39]. Similarly, incorporation of various minerals of Zamzam water into the enamel crystals as reported by Rawi *et al*.[38] could result in the increase in enamel density and volume in the current study.

Compared to the demineralized group, there was no significant difference ($p > 0.05$) between F and demineralized groups regarding enamel density after remineralization with a statistically significant increase ($p \leq 0.05$) of the two Zamzam gel groups over F group regarding effect enamel volume after remineralization indicating a week effect of fluoride gel in comparison with Zamzam gel (Z1 and Z2) groups. These results may be explained by the various mineral content of Zamzam water as calcium, sodium, magnesium, and fluoride[49]. Moreover, Zamzam water is a safe natural product that is suitable even in the form of gel to be used for several times. In contrast to APF gel which contains a higher amount of fluoride that make its use is limited to avoid its toxicity and was used in the current study only once per week[50].

Although we tried to mimic the oral environment by pH cycling, further clinical studies are required to emphasize the effect of Zamzam gel on remineralization of artificial caries. It is also mandatory to perform clinical studies on its application as a preventive measure before recommending its usage in dental practice.

CONCLUSION

Zamzam water in this new form was superior to fluoride gel and could compensate the surface irregularities induced by acid demineralization of enamel so our hypothesis was accepted. Thus, Zamzam water in gel form may be effective in the remineralization of the demineralized enamel surface and its effectiveness is superior to that of fluoride. This suggests the cariostatic potential of Zamzam gel and its ability to be used not only as a therapy but also as a preventive measure avoiding the risks of fluoride toxicity.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التقييم النسيجي وبالأشعة ثلاثية الأبعاد المخروطية لماء زمزم (هلام) مقابل الفلورايد الموضعي في إمكانية إعادة التمعدن في تسوس المينا المستحث (دراسة في المختبر)

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المقدمة: المنتجات الطبيعية أصبحت الهدف الجديد لطب الأسنان الحديث.
الهدف: تقييم تركيبة جديدة لماء زمزم (على شكل هلام) في إعادة تكلس التسوس الناتج صناعياً مقارنة بهلام الفلورايد.
المواد والطرق: تم توزيع مائة وعشرة ضواحك عشوائياً إلى خمس مجموعات متساوية (٢٢ سناً/مجموعة). تم إنشاء آفات تسوس اصطناعية على جميع الأسنان باستثناء المجموعة الأولى من الأسنان السليمة. تم تقسيم الأسنان المتبقية عشوائياً إلى: المجموعة الثانية (الضابطة السلبية): الأسنان التي تركت دون علاج. المجموعة الثالثة (مجموعة جل الفلورايد) (الضابطة الإيجابية): عولجت الأسنان بهلام فلوريد الفوسفات المحمض. المجموعة الرابعة (جل زمزم ١) والمجموعة الخامسة (جل زمزم ٢): عولجت الأسنان بهلام زمزم بتركيز عادي وتركيز عالي على التوالي. بعد ذلك، تم تقييم سطح المينا نوعيًا عن طريق المجهر الإلكتروني الماسح وكميًا من خلال تقييم الصلابة الدقيقة للسطح باستخدام اختبار فيكر للصلابة الدقيقة، ومن خلال مسح بالأشعة ثلاثية الأبعاد المخروطية لكثافة وحجم المينا.
النتائج: أظهر الفحص المجهري الإلكتروني الماسح لمجموعتي هلام زمزم ١ و٢ تحسناً واضحاً في سطح المينا منزوعة المعادن مقارنة بمجموعة الفلورايد. كما لوحظ وجود زيادة إحصائياً ($P \leq$ ٠,٠٥) في مجموعتي هلام زمزم على مجموعة الفلوريد فيما يتعلق بكثافة المينا وحجم المينا الفعال بعد إعادة التكلس، إلى جانب زيادة إحصائياً ($P \leq$ ٠,٠٥) في هلام زمزم ٢ على مجموعة الفلوريد ومجموعة هلام الفلورايد فيما يتعلق بالصلابة الدقيقة.
الاستنتاجات: يمكن أن يتفوق جل زمزم على جل الفلورايد مما يشير إلى القدرة على استخدامه ليس فقط كعلاج ولكن أيضًا كإجراء وقائي لتجنب مخاطر سمية الفلورايد.