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Research Article

Remineralization Potential of Zamzam Water on Eroded Primary Tooth Enamel: An In Vitro Study

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ABSTRACT: The current study aimed to investigate the influence of Zamzam water on the enamel of eroded deciduous anterior teeth using the surface microhardness test and a scanning electron microscope for morphological evaluation. **Materials and methods**: 40 deciduous incisors were used in the study. Blocks of enamel were prepared and immersed in Fanta cycle then divided into four groups (10) samples for each one: group (1) samples were kept in de-ionized water only, group (2) samples were treated with Sodium Fluoride solution (NaF), group (3) treated with Zamzam water while the samples of group (4) were treated with combined Sodium Fluoride plus Zamzam water. A Vickers microhardness tester was used to evaluate the microhardness of the enamel surface in all tested samples at baseline, after completion of the Fanta cycle, and after the treatment cycle, with confirmation of surface changes by scanning electron microscopy. The obtained results were statistically analysed. **Results**: Statistically significant differences were demonstrated among the tested groups using a one-way ANOVA test after treatment cycles. Duncan's multiple analysis of variance test was carried out to illustrate that no significant difference in surface microhardness readings was found among all groups at baseline. However, after treatment with different remineralizing agents, a significant difference in microhardness values was observed. Additionally, a scanning electron microscope revealed a rough, damaged surface after immersion in Fanta, but it recovered its smoothness after the treatment stage. **Conclusion**: The combination of NaF solution and Zamzam water demonstrated better demineralization resistance and improved surface characteristics, including surface microhardness, compared to NaF solution alone or Zamzam water alone. This combination also exhibited a beneficial anti-erosive effect on the eroded samples.

Keywords: Primary teeth; Erosion; Surface hardness; Zamzam water; Remineralization.

INTRODUCTION

Dental erosion, as one of the main causes of tooth tissue destruction, is a chemical dissolution of the teeth structures due to contact with acids whose pH is lower than 4.5 [(1)].

In the last several decades, the consumption of commercial soft drinks has dramatically increased up which are high in acidity and sugar content; therefore, their consumption contributes significantly to being deleterious to oral and general health. Carbonated beverages are dominant among these drinks; the increased consumption of carbonated beverages is correlated with an increase in erosive damage to organized enamel structure [(2)].

There are many measures of preventive programs that have been proposed by researchers for controlling enamel erosion, and the use of Fluoride (F) is one of them [(3)]. On the other hand, unfortunately, F fails to guide the formation of new mineral crystals and to form organized and oriented crystals on the enamel surfaces [(4)]. Therefore, attention has been centered on discovering and using new beneficial natural products that have many advantages with minimal side effects as Zamzam water.

Zamzam water is an alkaline, natural, carbonated, and highly mineralized water with extraordinary physical and chemical characteristics that differ from any other water. For cure or for religious belief, a lot of individuals drink Zamzam water [(5)]. Chemical laboratory tests showed that Zamzam water is absolutely safe to drink and has health benefits because of its contents of Calcium, Sodium, Fluoride, Magnesium, Potassium, Bicarbonate, and several other minerals, higher than other water types [(6)]. As Zamzam water exhibits high levels of minerals, consequently, it can be introduced as a means for the prevention of dental erosion in the deciduous and permanent dentitions [(7)].

MATERIALS AND METHODS

The protocol of this study was conducted in vitro, submitted, and approved by the Local Ethics Committee (UoM.Dent/ H.DM.75/ 22) Research Ethics Committee of the College of Dentistry, University of Mosul, Nineveh, Iraq.

Teeth sample collections and preparations

Following the ethical committee permission, 40 extracted deciduous incisors (A or B) with intact enamel surface (no developmental anomalies, no caries, cracks, stain, fluorosis, previous restorations) were collected due to over retention or physiologic mobility from a child patient between the ages of 6-7 years from private clinics of Mosul city.

All the targeted teeth were cleaned, deposits were removed, then rinsed with de-ionized water and stored in a thymol solution 0.1 % as an antimicrobial agent [7]. Polishing the teeth with non-fluoridated pumice using dental polishing rubber cups was done before detaching the roots from the crowns. Separation of crowns from roots with the level of cutting 2mm below the cementoenamel junction [8]. A cylindrical plastic molds were previously designed to receive the prepared teeth [9] by embedding them in the center of the molds filled with chemically polymerized acrylic resin and exposing the labial surfaces upward [8]. For standardization of the exposed surfaces to the microhardness test, wet silicon carbide grit papers with grinding sizes 400, 600, 800, and 1000 were used for 30 seconds for each grain size for the production of flat enamel surfaces free from scratches to make them ready for initial (baseline) microhardness measurements [10].

The Design of the Study

Fanta Immersion Cycle

After baseline measurement of surface microhardness values, all teeth were submerged in undiluted Fanta drink for 5 min (1x3) for 6 days at room temperature [11]. The Fanta drink should be replaced before each use. After each cycle of immersion, the samples were washed with distilled water and preserved in it. The solution should be changed daily to avoid the accumulation of substances formed through demineralization [8].

Grouping

After the demineralization cycle by Fanta, the 40 deciduous teeth were randomly split into 4 groups for treatment cycles as below, with 10 samples in each group:

1. Group 1: Control negative group: these samples were kept in de-ionized water.

2. Group 2: Control positive group: these samples were immersed in a 0.05% NaF solution for 4 minutes daily for 7 days [12].

3. Group 3: Study group: the samples were exposed to the Zamzam water for 30 min every 12 h for 12 days, and after each cycle, the teeth were washed for 2 min with de-ionized water and kept in it until the next cycle [7].

4. Group 4: Combination group: these samples were exposed to the NaF solution for 4 min, washed, and kept in de-ionized water for an hour, then followed by 30min immersion in Zamzam water, and after 12h, another Zamzam water cycle was applied. Subsequent to treatment cycles, all samples were subjected to microhardness values recorded by the Vickers hardness test.

Surface Microhardness Test

This test was done at the Technical Institute/North Technical University by a Vickers hardness machine (OTTO Wolpert, V-Tester 2, Germany) as shown in Figure 3.20. In

each sample, an area was marked with nail varnish in the middle of the buccal and labial surfaces. Serially, three indents were made using a microhardness indenter in a straight line [13] with 500 g of force loads for 15 seconds for each indent (as stated by the instruction of the machine's manufacturer). Hardness measurements were performed using a 600× magnification with a square-based diamond pyramidal tip featuring a 136º angle, which appears on the resulting sample as a rhomboidal shadow [14]. Vickers hardness number (VHN) is calculated microscopically, measured in microns from the size of the impression produced on the enamel surfaces for a specified length of time by a pyramidal-shaped diamond indenter, and this can be done firstly through the application of the following formula:

VD + HD = TD where:

VD is the vertical diagonal length.

HD is the horizontal diagonal length.

TD is the total diagonal length measurement.

Then, the mean value of the TD length measurement of the three indentations was calculated and reported as one reading [15].

Finally, Vickers values were converted into microhardness values by the following equation:

HV = 1.854 (p/d2) where:

HV= microhardness value in Kg/mm² (MPa).

P= the testing load measured in grams.

d= d1+d2 /2 [16].

Scanning Electron Microscope for Morphological Evaluation (SEM)

Scanning electron microscope observations carried out at the University of Ninavah, College of Pharmacy. A special block (8 mm height) of the specimens was embedded in auto polymerized cold-cure acrylic resin [17]. Teeth were cleaned, polished and immersed in Fanta drink (5 min 3 times daily for 6 days) then divided at random to groups; the control -ve group (which kept in de-ionized water), the control +ve group (which exposed to 0.05% NaF solution 4 min daily for 7 days), the study group (exposed to the Zamzam water for about half an hour every 12 h for 12 days) and lastly the combination group (exposed to the 0.05% NaF solution 4 min washed and kept in de-ionized water for an hour then followed by 30min immersion in Zamzam water and after 12 h another Zamzam water cycle applied).

Each tooth was coated with a pure gold layer 10 nanometer in thickness for about 1 min under a vacuum condition using Argon gas with pressure of 10-2 bar by a sputtering coater device (Quorum, Q300T T, USA) as shown in figure (1) so that the surface morphological changes of enamel to be observed obviously with the SEM

under 8 and 10 kv accelerating voltage. The morphology of the enamel surfaces was examined, and representative photomicrographs were taken and stored digitally [18].

Figure (1): Sputter coater device (Quorum, Q300T T, USA).

Statistical Analysis

The experimental data were analyzed statistically through utilizing the statistical package for social sciences software program (IBM SPSS Statistics 26), which includes; Shapiro-Wilk test to examine the type of distribution of the data, and which was normally distributed as shown in Table 1. Also, one-way ANOVA and Duncan tests help us to decide whether there are any statistically significant differences or not among the groups.

RESULTS

Table (1): Shows the test of normality for teeth samples at baseline.

Groups	Shapiro-Wilk		
	Statistic	Df	Sig.
Control -ve	.906	10	.265
Control +ve	.903	10	.237
Study	.937	10	.520
Combination	.893	10	.185

Microhardness results

Statistical analysis in Table 2 describes the microhardness values by standard deviations, means, minimum, maximum, as well as the number of samples that have been tested in each group at baseline, after Fanta, and treatment cycles.

According to the mean values, all groups fall in their hardness after the demineralizing cycle protocol with Fanta as compared with that at baseline, then these values raised after the remineralization cycle, except for the control -ve group, which kept the same value (218.34) of demineralization as these samples of teeth were not exposed to any of the remineralizing agents. By the way, the maximum increase in the enamel surface microhardness belonged to the combination group, followed by the control +ve, then the study groups of Zamzam water.

Table (2): Descriptive statistics of microhardness measurements for deciduous teeth at baseline, after Fanta, and treatment cycles.

Stage	Groups	N	Minimum	Maximum	Mean	Std. Deviat
Baseline	**Control -ve**	10	215.58	299.00	250.26	29.56894
	Control +ve	10	201.50	343.33	259.39	38.49011
	Study	10	197.23	299.00	251.41	26.89087
	Combination	10	201.50	331.00	265.72	49.50763
After cycle	**Control -ve**	10	157.10	257.50	218.34	37.33051
	Control +ve	10	171.60	257.50	214.71	34.10345
	Study	10	176.00	257.50	218.41	25.16835
	Combination	10	162.63	289.68	212.15	47.06151
After treatment	**Control -ve**	10	157.10	257.50	218.34	37.33051
	Control +ve	10	234.00	280.00	252.76	13.98332
	Study	10	201.50	299.00	249.86	32.41389
	Combination	10	197.23	309.00	256.74	37.76843

In Table (3), the one-way ANOVA test for deciduous teeth shows that there was a significant difference among the tested groups in surface microhardness readings at $p \leq 0.05$.

Table (3): One-way ANOVA test among the examined groups at $p \leq 0.05$.

Microhardnes	Sum of Square	DF	Mean of Square	F	Sig.
Between Grou	9307.595	3	3102.532	3.052	.041*
Within Group	36595.931	36	1016.554		
Total	45903.527	39			

No significant difference among the tested groups of deciduous teeth at baseline was confirmed by using Duncan multiple analysis test, where all data were organized in a homogenous subset representing the surface microhardness mean values of each group, as shown in Table (4).

Table (4): Duncan multiple analysis range test for the examined groups at the baseline stage.

		Subset for alpha = 0.05
Groups	**N**	1
Control -ve	10	250.263
Study	10	251.419
Control +ve	10	259.399
Combination	10	265.722
Sig.		.405

But after treatment application, there is a maximum raised in hardness values belonging to the union of Zamzam water plus NaF remineralizing agents in group (4), that increase in the combination group followed by the increase of the Control +ve group of NaF solution, and then by the study group, as displayed in table (5).

Table (5): Duncan multiple analysis range test for after after-treatment stage.

		Subset for alpha = 0.05	
Groups	**N**	**1**	**2**
Control -ve	10	218.348	
Study	10		249.859
Control +ve	10		252.758
Combination	10		256.745
Sig.		1.000	.653

SEM

Morphological changes in the enamel surface after application of different treatment strategies were evaluated using SEM (ZEISS, version 7.01, Germany).

At baseline

Initial SEM examination of the enamel samples at baseline manifested a flat smooth surface with a lack of visible surface cracks in the untreated polished enamel, as shown in Figure (2).

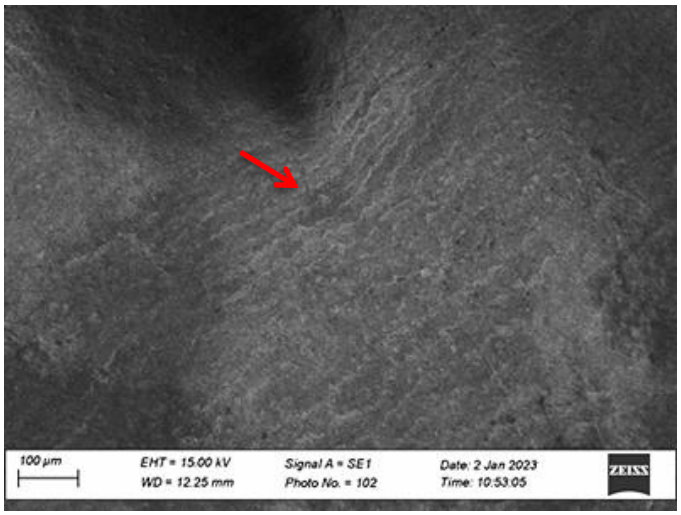

Figure (2): SEM images of the enamel surface at baseline showed normal, intact, and no irregularities with clear perikymata (incremental growth lines) that can be represented by red arrows with a magnification time used 200x.

After Fanta Immersion

Scanning examination of the labial surfaces revealed eroded enamel surface with some areas having destroyed prism cores but with definite prism boundaries giving the characteristic of unorganized structure, while other areas were covered by rodless enamel, as in Figure (3).

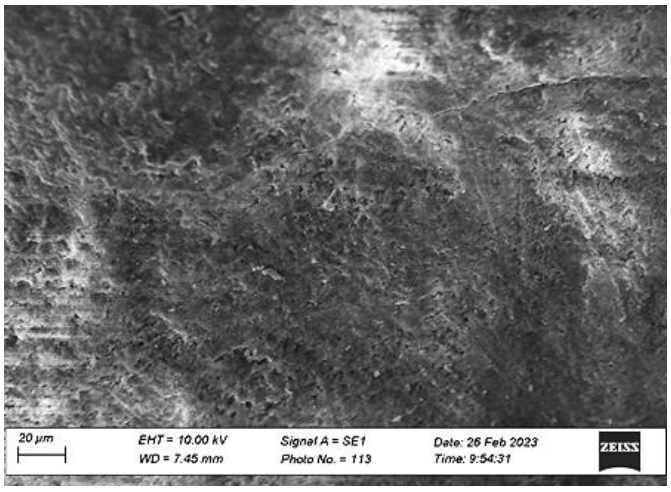

Figure (3): The samples exhibited a rough surface, and opened enamel rods became more advent due to erosion by the action of the acid at 1000x magnification.

After Treatment:

Control +ve Group

Samples that were treated with NaF solution showed a surface layer with globular material precipitations of CaF_2 that can be seen in Figure (4).

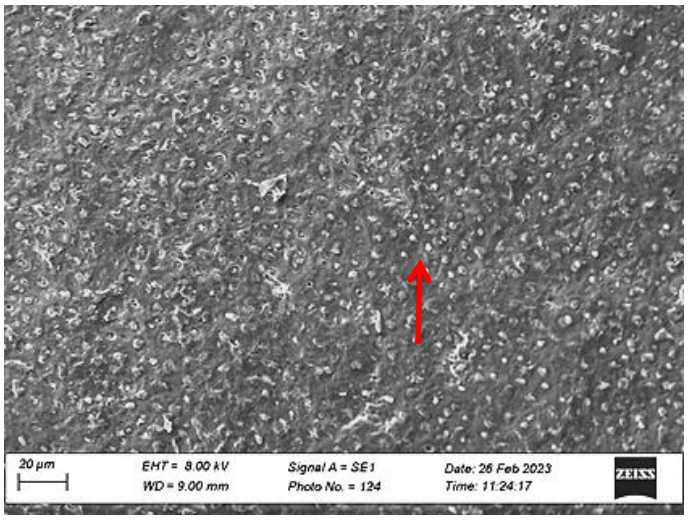

Figure (4): Examination of samples at 1000x magnification of the control +ve group, manifestation of CaF_2 globules, which were presented by red arrows.

Study Group

Zamzam water samples in Figure (5) showed a pronounced repair of the demineralized surface under SEM.

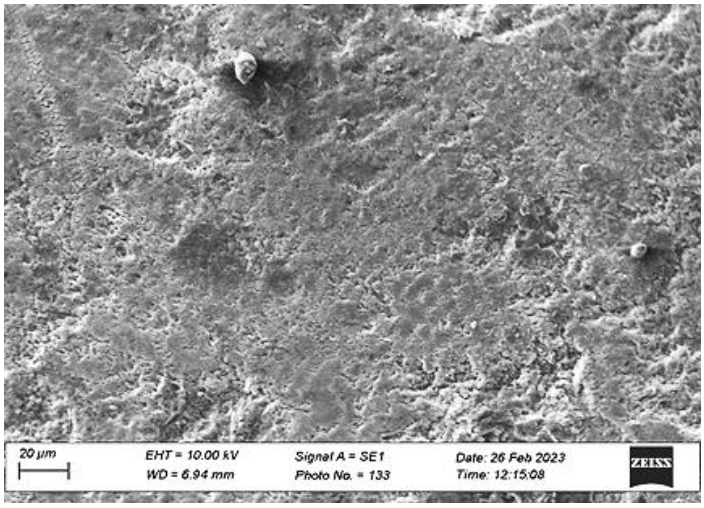

Figure (5): The SEM image shows that recovery of surface smoothness to some extent can be noticed when a magnification power used 1000x.

Combination Group

In Figure (6), Samples that were treated with a combination protocol of NaF solution plus Zamzam water showed a great enhancement of surface flatness, especially with the disappearance of exposed enamel rod ends.

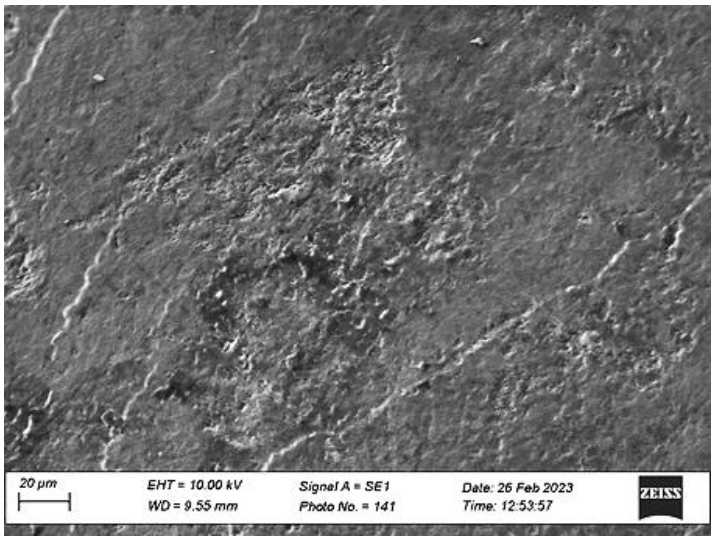

Figure (6): A Uniform and harmonious enamel layer covered the previously eroded enamel surfaces, magnified at 1000x.

DISCUSSION

Dental erosion is a global and popular problem in the dentistry field. Thus, dental erosion and its subsequent tooth damage because of chemical agents that cause loss of tooth mineral by the attack of acids without interfering with bacterial development and activity are a growing problem in modern societies. The consumption of non-alcoholic commercially prepared carbonated beverages is one of the external etiological factors that cause dental disintegration or wearing [19]. A survey in Mosul city by AL-Dahan and Al-Jobory (2006) revealed a highly carbonated drink intake among 27 primary schools, with the prevalence of different percentages of erosion [20]. Fanta is an American-owned German brand of fruit-flavored carbonated soft drinks. Its erosive effects are attributed to citric acid of pH = =3 That can chelate calcium, reduce the buffering capacity of saliva, and so increase the destruction rate of the tooth body [21]. According to the results of the existing study, after soaking the deciduous and permanent teeth in the Fanta beverage (demineralized solution), there was a large reduction in the microhardness and increased surface roughness of the enamel surface in all tested groups. The findings of the present study were in agreement with the study of Ajami *et al*. (2006), who also used the Vickers hardness method, and their results showed a significant reduction of microhardness in tooth enamel after

immersion in beverages [22]. Also, another study demonstrated that acidic beverages have an impact on the reduction of the hardness of teeth and result in erosion of the tooth [23]. In SEM, as the samples were subjected to a demineralization cycle by Fanta drink, the surfaces revealed a lot of disorganization with extensive areas of defects caused by erosion as evidence of generalized structural loss, in addition to opened enamel prisms could be detected.

A natural remineralizing material as a primary prevention of dental erosion and caries has been conducted in the current study to assess its impact on deciduous teeth. For this reason, 0.05% NaF solution was chosen in the present experiment as a positive control with the same protocol of application as administered by Al-Lami and Al-Alousi (2010) to evaluate its inhibitory action of demineralization and promotion of surface remineralization by microhardness measuring [24]. The recorded microhardness values of this group revealed a statistically significant increase when compared to demineralization values after Fanta. Thus, Sivapriya *et al*. (2017) recommended that the repeated application of NaF can improve the microhardness of demineralized dental tissues on enamel and dentin [25]. The SEM image of the enamel surface, which was treated with NaF solution (Control +ve), shows the formation of a dense coating layer of numerous granular particles of different sizes of CaF_2, which occlude the previously opened rods.

In the present study, Zamzam water was also successful in the increase of the microhardness value of demineralized deciduous and permanent enamel surfaces as found in the study group. This may be attributed to the localization of calcium and phosphate ions with the Fluoride ions at the outer enamel surfaces, which exist in Zamzam water with high levels and form the major components of apatite crystal [26]. As supporter evidence for the results of the current study, the primary central teeth included in a study done by Kufiyah *et al*. (2021) showed a significant rise in their surface microhardness after Zamzam water treatment by more than 150% [7]. In confirmation of our study's results in SEM, in spite of a few areas of un full recovery of flatness, Zamzam water offered special and pronounced repair of surface defects. In agreement with the SEM results of the present study, Elkabbany *et al*. (2022) stated advanced results of the Zamzam water group after remineralization, achieving a significant calcium and phosphorus gain up to 6.04% for calcium and 7.9% for phosphorus [27].

The hardness results of enamel samples of the combined group of NaF plus Zamzam water presented with highest elevation among the other groups, which was an indication of the double action of Fluoride ions and the notable impact of Ca^{+2} and PO_4^{-3} ions in rebuilding the destroyed crystals. The samples had an orderly formed enamel

layer due to the double action of NaF plus Zamzam water, which appeared to be the most effective in protecting enamel erosion when combined compared to other groups.

CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that:
Fanta soft drink showed an erosive effect reflected by decreasing surface microhardness, which is verified by SEM. Zamzam water, in addition to traditional fluoride solution (NaF), showed good remineralization effects on deciduous teeth. Therefore, it could be introduced as part of the prevention and treatment of dental erosion in children. The union of NaF plus Zamzam water in the combination group observed a better remineralization effect, including the maximum increase in microhardness values.

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Authors' Contribution

Al-Taiee HT., Qasim AA contributed to conceptualization, validation, and writing the original draft. Al-Taiee HT was responsible for formal analysis, methodology, and project administration. Qasim AA contributed to supervision, review & editing of the manuscript. Al-Taiee HT., Qasim AA contributed to the investigation, software, validation, and visualization. Al-Taiee HT was involved in data curation, resources, and review & editing. All authors have read and approved the final manuscript.

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Ethical statement: The protocol of this study was conducted in vitro, submitted, and approved by the Local Ethics Committee (UoM. Dent/ H.DM.75/ 22), Research Ethics Committee of the College of Dentistry, University of Mosul, Nineveh, Iraq.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Availability of data and materials: Data is available at the request of the corresponding author.

Declaration of Generative AI and AI-assisted technologies

No generative AI or AI-assisted technologies were used in the preparation of this work. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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إمكانية إعادة تمعدن ماء زمزم على مينا الأسنان الأولية المتآكلة: دراسة مخبرية

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2 قسم طب أسنان الأطفال، تقويم الأسنان، طب الأسنان الوقائي، كلية طب الأسنان، جامعة الموصل، الموصل، العراق

الملخص

الأهداف: تهدف الدراسة الحالية إلى مقارنة وتقييم تأثيرات ماء زمزم ومحلول الفلوريد الكلاسيكي على الصلابة الدقيقة السطحية وخشونة عينات الأسنان اللبنية و الدائمة المتآكلة في المختبر .**المواد وطرائق العمل:** تم استخدام مجموعه (80) من الأسنان السليمة التي تم خلعها حديثاً. 40 عينة للأسنان الأمامية اللبنية و 40 عينة للضواحك الدائمة في الدراسة. تم تحضير عينات اسطح المينا لكل نوع من الأسنان وتعريضها لدورة فانتا ثم قسمت عشوائياً إلى أربع مجموعات كل منها تضم 10 عينات: المجموعة السالبة والتي تم حفظها في الماء غير المتأين فقط. تم غمر المجموعات الثلاث الأخرى في مواد إعادة تمعدن مختلفة على النحو التالي: مجموعة السيطرة الموجبة (محلول صوديوم فلورايد) ، ومجموعة الدراسة (ماء زمزم) والمجموعة المركبة (صوديوم فلورايد مع ماء زمزم). تم اختبار الصلابة السطحية الدقيقة وخشونة المينا قبل وبعد دورة الفانتا وبعد دورة إعادة التمعدن باستخدام جهاز قياس الصلابة الدقيقة من فيكرز وآلة قياس الخشونة بمقياس بروفيلومتر. **النتائج:** إنخفضت الصلابة الدقيقة للأسطح لجميع المجموعات المختبرة بعد التآكل باستخدام مشروب فانتا بسبب سلسلة فقدان التمعدن ، ثم زادت بعد العلاج بمختلف مواد إعادة التمعدن. كانت أعلى زيادة في قياسات الصلابة السطحية في كل من

الأسـنان اللبنية والدائمة تنتمي إلى مزيج محلول صـوديوم فلورايد + مجموعة ماء زمزم تليها مجموعة صـوديوم فلورايد ثم مجموعة ماء زمزم ، بينما لم تتحسـن صـلابة المجموعة السـالبة من الماء غير المتأين. كما زادت خشـونة السـطح لجميع مجموعات الدراسة في كلا نوعي الاسنان بعد التآكل بشراب فانتا ، ثم إنخفضت بعد المعالجة بمواد إعادة التمعدن ، مع تحسن شـبه كامل في نعومة السـطح (انخفاض في خشـونة السـطح) في المجموعة المركبة من صـوديوم فلورايد مع ماء زمزم تليها مجموعة صـــوديوم فلورايد ثم مجموعة ماء زمزم ، بينما لم يكن للماء غير المتأين أي تأثير لإعادة تمعدن عينات الأســنان .**الاسـتنتاجات:** كان تركيبة محلول صـوديوم فلورايد مع ماء زمزم الأفضـل في مقاومة فقدان التمعدن وتحسـين خصـائص السـطح بما في ذلك الصـلابة السـطحية الدقيقة وخشـونة السـطح للمينا المتعرجة اللبنية والدائمة من محلول صـوديوم فلورايد وحده أو ماء زمزم وحده والذي كان له أيضًا تأثير مفيد مضاد للتآكل على عيناتهم.

الكلمات المفتاحية: الأسنان اللبنية؛ التآكل؛ صلابة السطح؛ ماء زمزم؛ إعادة التمعدن.