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Research Article

The Effect of Zamzam Water and ProShield[R] Varnish on Microhardness of Eroded Primary Enamel Teeth Induced by Iron[plus] Syrup: An *In Vitro* Study

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ABSTRACT

Background: The objective of the current study was to evaluate the erosive adverse effect of paediatric Iron[plus] syrup and the remineralization effectualness of Zamzam Water and ProShield[R] Varnish on microhardness of primary teeth. **Materials and method:** Forty primary anterior teeth were randomly distributed into five groups, each group has eight samples (N=8) assigned as: group1: control negative, group2: control positive, group3: 5% Sodium Fluoride Varnish, group4: Zamzam water and group5: Zamzam water and 5% Sodium Fluoride Varnish. Excluding the control negative group from them, all samples were immersed in 100ml of Iron[plus] syrup and agitated for 2 min once a day for 14 days. Then those groups were treated with remineralizing agents of: 5% Sodium Fluoride Varnish for 24 hours, Zamzam water for 30 min every 12 h for 12 days and the combination therapy initiated by Zamzam water then after 30 min, 5% Sodium Fluoride Varnish was applied for 24hrs. Vickers Microhardness test and Polarized Light Microscope were two adopted tests that this study dependent upon. **Results:** There was a statistically significant difference in microhardness mean values when making a comparison between the baseline and Iron[plus] tested groups, furthermore ANOVA and Duncan's Multiple Range test showed that there was a highly significant statistical difference in microhardness mean values among baseline, demineralization and remineralization groups at P-Value ≤ 0.01. **Conclusion:** The surface microhardness of the primary enamel was reduced owing to the potential erosive efficacy of the used paediatric pharmaceutical preparations. On the other extreme, the previous readings had increased after being treated with remineralizing agents meaning that they have the capacity to battle against erosive potential reaching its highest capability in terms of reminerlization with the combination treatment group.

Keywords: Erosion, Iron[plus] Syrup, Zamzam water, ProShield[R] Varnish, Microhardness, Polarized Light Microscope.

INTRODUCTION

The most prevalent dietary deficits worldwide are an iron deficiency.[1]. Anemia caused by iron deficiency is a serious health problem that impacts both developed and developing nations globally.[2] Hemoglobin (Hb) levels of less than 13 g/dl for men and 12 g/dl for women are considered iron deficiency anemia by the World Health Organization (WHO).[3] Iron deficiency and anemia were quite common all across the globe, but particularly in Africa and Asian countries.[4]

Humans with IDA or those seeking iron supplements can purchase commercial iron supplements. Typically, it is administered to children between the ages of 6 months and 2 years old to prevent iron deficiency.[5] The first line of treatment for people with iron insufficiency and iron deficiency anemia is oral iron supplementation. Oral iron administration is practical, affordable, and generally efficient.[6] Due to their ease of administration, patient compliance with therapy, and ease of quickly modifying dosages throughout treatment based on the pathology and child development, liquid solutions and suspensions are the most suitable ones for use in pediatrics.[7] While the active chemicals in these medications are essential for maintaining or restoring health, some inactive ingredients may be harmful, such as those that promote dental erosion and caries. Dental erosion is characterized by the continuous chemical degradation of the hard tissue of the teeth without the presence of microorganisms; it is a complex and multifaceted phenomenon that is permanent in nature and may be affected by internal, external, or intrinsic factors.[8] Extrinsic type of dental erosion could result from any drug with a low pH that comes into touch with teeth often and/or for an extended period of time.[9] To potentiate the absorption of iron in a positive manner, water-soluble Vitamin C, also referred to as Ascorbic Acid, are usually obtained from the diet.[10,11] It has a low pH and a high titratable acidity.[12] children who take vitamin C supplements tend to have erosive lesions 4.7 times than their peer groups.[13] Early recognition, understanding of the etiologies and risk factors for erosion form the basis of a diagnostic protocol and management strategy, otherwise irreversible dental deteriorations in terms of dental sensitivity, occlusal loss, poor oral health, pulp exposure, and abscess would be evident.[14]

The elixir of life is water.[15] It is an integral nutritional element.[16] It is important for survival and is crucial for many bodily physiological processes.[17] It serves as a carrier and solvent while food's vitamins and other critical nutrients are dissolved and transferred to cells.[18] Although there are few sources of water and those that do exist are gradually running out, there is one source of water known as This spring is exceptional in that it never runs out or becomes dry

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and always flows profusely.[19] A water well called Zamzam can be found in the Valley of Abraham in the Saudi Arabian holy city of Makkah. The well is currently on the ground floor and is encircled by glass plates, allowing visitors to get a look into clearly.[20] Due to the presence of several cariostatic components as fluoride, calcium, sodium, and potassium, Zamzam water is helpful in decreasing enamel solubility and increasing its resistance to caries attack.[21] The hydroxyapatite crystals in teeth conduct chemical reactions with all of these minerals, which reduce porosity and promote enamel microhardness.[22] When it comes to remineralizing initial carious lesions in permanent teeth, Zamzam water is just as effective as sodium fluoride 0.05%.[23,24] Consequently, dental enamel's microhardness was raised.[25] Zamzam Water has a high content of bicarbonates, which is an essential element in saliva that helps to balance out the pH of the mouth. It is essential to gargle with water after meals in order to maintain oral clearance due to bicarbonate content.[26]

Fluoride varnish application should start from the time of primary teeth eruption. Varnishes are painted directly on the teeth by the dental care practitioner.[27] Fluoride varnish is applied to teeth to form a thin film that remains on the teeth for a long time, and thereby provides sustained fluoride release, it is maintained in the oral cavity for 1-7 days after application.[28] When fluoride is introduced to the oral cavity from topical sources, it is incorporated into the salivary pellicle. Calcium ions in plaque fluid attract fluoride ions, ultimately facilitating fluoride to adsorb to the enamel surface. Fluoride is incorporated into the demineralized surface, forming a fluorapatite crystal.[27] Fluorapatite has a lower solubility in solution than hydroxyapatite, making the remineralized portion of enamel more resistant to acid challenges.[29] The professionally applied topical fluoride varnish is the easiest and quickest as it takes only 1-4 minutes for its application.[30]

MATERIALS AND METHODS

Sample Collection and Preparation: Primary anterior teeth of (6-8) years old children were collected from the pediatric dental clinic in specialized dental center in Mosul. Forty primary anterior teeth were randomly chosen as a sample. Before use, teeth were washed thoroughly under running tap water to remove blood, saliva and debris Using a stereomicroscope, the condition of primary anterior teeth was investigated to rule out abnormalities including dental caries, cracks, and fractures. The roots were cut at the level of the cement-enamel junction (CEJ) using a straight diamond bur of a high-speed handpiece with continuous water cooling. The coronal sections of the specimens were then implanted in auto polymerized cold cure acrylic resin blocks utilizing cylindrical plastic tubes (15 mm in height). Before employing the surface microhardness test, the enamel surfaces were polished using a universal polisher machine as a final step for better visualization of surface indentation on tested teeth.

Study design

Group 1: Control negative group No. = (8), these samples were kept in deionized water.

Group 2: Control positive group No. = (8), these samples were exposed to pH- cycling (Iron [plus] syrup) only.

Group 3: No. = (8), after immersion of teeth samples in Iron [plus] drug cycle, the enamel surface was treated with ProShield[R] varnish.

Group 4: No. = (8), after the immersion in Iron[plus] drug cycle, the enamel surface was treated with Zamzam water.

Group 5: No. = (8), after the immersion in Iron [plus] drug cycle, the enamel surface was treated with combination of Zamzam water and ProShield[R] varnish.

Immersion Cycle: The teeth were immersed into 100 ml of undiluted syrup and agitated for 2 min once a day for 14 days. [31]. After each immersion, the teeth were washed with distilled water and preserved in deionized water with daily change of the solution. The medicines were replaced before each immersion and at the end of 14 days teeth were transported to microhardness testing laboratory, to evaluate their microhardness status.

Treatment Cycle: For third group, the enamel surface will be treated by sodium fluoride varnish 5% (the enamel surface will be coated by a fine brush with a thin layer of sodium fluoride varnish 5%) for 24 hrs. and then will be washed for 1 min with deionized water.[32] For forth group, the enamel surface will be treated with Zamzam water for 30 min every 12 h for 12 days. After each cycle in Zamzam water, the teeth will be washed for 2 min with deionized water and kept in deionized water to the next cycle.[33] to avoid dehydration and the changes in the microhardness.[34]

For fifth group, the enamel surface will be treated firstly with Zamzam water for 30 min every 12 h for 12 days and kept in deionized water then after 30 mins, the enamel surface will be treated by sodium fluoride varnish 5% for 24 hrs and then will be washed for 1 min with deionized water.[32]

Chemical Analysis of Zamzam's Water Mineral Profile: Calcium and Magnesium were determined by Complexometric titration while Phosphate by Spectrophotometer and Fluoride by Fluoride Ion-Selective Electrode (ISE). These procedures were done in College of Agriculture and Forestry at Mosul University.

Vickers Microhardness Test: The Vickers microhardness machine was used at Mosul Technical Institute/North Technical University to measure the microhardness of the enamel surface of the teeth.

Vickers hardness number is a function of the known applied force divided by the indentation's surface area. Throughout the whole investigation, which was conducted by the same examiner, utilizing the same calibrated machine, a load of 500gm was maintained extended between 10-15 seconds at a steady rate for all samples, carried out at room temperature and in a dry environment. Since the enamel surface has a curve, the indentations were done at a magnification of 70x and recorded as 3 distinct indentations. Vickers microhardness is calculated as 1.8544 (p/d^2), where P is the applied stress measured in kilograms and d is the mean diagonal length, measured in millimeters so that microhardness value is in Kg / mm^2 (Mpa).[35]

Polarized Light Microscope: Polarized light microscopy (PLM) was used to assess demineralized lesions histologically and measure their depth[36], because it is incredibly sensitive to minute changes in the optical characteristics, it was employed in conjunction with microhardness studies for qualitative and quantitative evaluation of the lesion changes. The visualization of areas with various porosities and birefringence can qualitatively demonstrate the areas of mineral loss and mineral gain, which is the most accurate approach for calculating demineralization and remineralization.[37]

RESULTS

The chemical estimation of inorganic ingredients including Calcium, Magnesium, Phosphate and Fluoride of Zamzam water sample which reveals a high levels of ions concentrations in ppm or mg/l units (Table 1).

Paired sample t test was performed to conclude that there was a highly statistically significant different in microhardness mean values at baseline and after exposure to Iron[plus] cycle (Table 2).

The mean microhardness values of primary enamel samples were not significant prior to any treatment. On the other hand, after exposure to Iron[plus] medicinal syrup, an observable reduction in the mean values regarding surface microhardness of primary enamel compared to their

Table 1: Chemical analysis of Zamzam water.

Element's Name	Concentration (ppm or mg/l)
Calcium	60 ppm
Magnesium	48 ppm
Phosphor	0.02 ppm
Fluoride	1.35750 ppm (-424.9mV)

Table 2: Baseline and Iron[plus] After Cycle for microhardness using Paired t-test.

Microhardness	Mean	t-value	Sig.	N	Std. Deviation	Std. Error Mean
Baseline	335.7125	14.396	0.0001**	32	2.80247	0.49541
Iron[plus] After Cycle	257.1906			32	30.72727	5.43187

** Highly Significant at P-Value ≤ 0.01

Table 3: The descriptive statistics of microhardness measurements of Iron[plus] subgroup (at baseline, after Iron[plus] cycle and after treatment).

Descriptive Statistics						
Subgroup	Test	N	Min.	Max.	Mean	SD
Microhardness of Ironplus Baselines	Control Negative	8	330.01	342.83	336.420	6.85258
	Control Positive	8	334.28	336.28	335.280	0.53463
	Varnish	8	335.78	337.88	336.780	0.77469
	Zamzam	8	324.32	344.29	334.290	5.36420
	Combination	8	335.47	337.51	336.500	0.76371
Microhardness of Ironplus After Iron Cycle	Control Negative	8	330.01	342.83	336.420	6.85258
	Control Positive	8	257.30	306.25	288.270	16.44138
	Varnish	8	178.82	275.56	241.9487	29.62314
	Zamzam	8	233.56	306.45	256.5950	22.81654
	Combination	8	178.82	275.56	241.9487	29.62314
Microhardness of Ironplus After Treatment	Control Negative	8	330.01	342.83	336.4200	6.85258
	Control Positive	8	257.30	306.25	288.2700	16.44138
	Varnish	8	275.56	342.83	313.7875	27.98891
	Zamzam	8	275.56	317.90	309.7450	14.74957
	Combination	8	306.45	342.83	322.7838	14.44724

counterparts' baselines in terms of demineralization. However, the primary enamel regained its hardness property after being exposed to combination therapy which was (322.7838) followed by sodium fluoride group that was (313.7875) and the third one stands for Zamzam water remedy represented by (309.7450), these values belong to remineralization (Table 3).

ANOVA test revealed that there was no significant difference among baseline group since their values were very close to each other numerically and this was evident in Duncan[a] test. However, there was a statistically significant difference that co-existed among the three treated groups, control positive and control negative groups at $p \leq 0.01$, this means that although the microhardness of primary teeth had improved in different levels, none of the three treated groups had reached the control negative group level in addition to the control positive group that tends to minimize enamel hardness greatly due to the powerful acidity that this medicament hold (Table 4 and Table 5).

The utility of a specific equation which is SML%= SMH2 –SMH1 / SMH1 × 100, make it possible to calculate the loss of surface microhardness in percentage.

The least percentage of loss of surface microhardness was for the combination treatment group, followed by ProShield[R] varnish group, Zamzam water group and greatest for control negative of deionized water (Table 6).

DISCUSSION

Based on the mean vales, the result of this study found that the remineralizing effect was the best in the group treated with Varnish

Table 4: Microhardness of Iron[plus] at Baselines, After Iron Cycle, after treatmen using ANOVA test.

Microhardness of Iron[plus] – Baselines					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	34.966	4	8.741	0.566	0.689
Within Groups	540.412	35	15.440		
Total	575.378	39			
Microhardness of Iron[plus] *After Iron Cycle*					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	51621.975	4	12905.494	24.886	0.0001**
Within Groups	18150.528	35	518.587		
Total	69772.503	39			
Microhardness of Iron[plus] *After treatment*					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	10078.330	4	2519.582	8.250	0.0001**
Within Groups	10688.498	35	305.386		
Total	20766.828	39			

Microhardness, Subgroup = Microhardness of Ironplus – *Baselines*
** Highly Significant at $P \leq 0.01$

Table 5: Microhardness of Iron[plus] at Baselines, After Iron Cycle, After treatmen using Duncan test.

Microhardness of Iron[plus] – Baselines					
Test	N	Character	Subset for alpha = 0.05		
			1		
Zamzam	8	A	334.29		
Control Positive	8	A	335.28		
Control Negative	8	A	336.42		
Combination	8	A	336.5		
Varnish	8	A	336.78		
Microhardness of Ironplus *After Iron Cycle*).					
Test	N	Character	Subset for alpha = 0.05		
			1	2	3
Varnish	8	A	241.9488		
Combination	8	A	241.9488		
Zamzam	8	A	256.5950		
Control Positive	8	B		288.2700	
Control Negative	8	C			336.4200
Microhardness of Ironplus *After Treatment).*					
Test	N	Character	Subset for alpha = 0.05		
			1	2	3
Control Positive	8	A	288.2700		
Zamzam	8	B		309.7450	
Varnish	8	B		313.7875	
Combination	8	BC		322.7838	322.7838
Control Negative	8	C			336.4200

Table 6: Surface microhardness loss percentage for Iron[plus] subgroup.

Groups	SML%
Deionized water	14.31%
ProShield[R] varnish	6.72%
Zamzam water	7.92%
Zamzam water and ProShield[R] varnish	4.05%

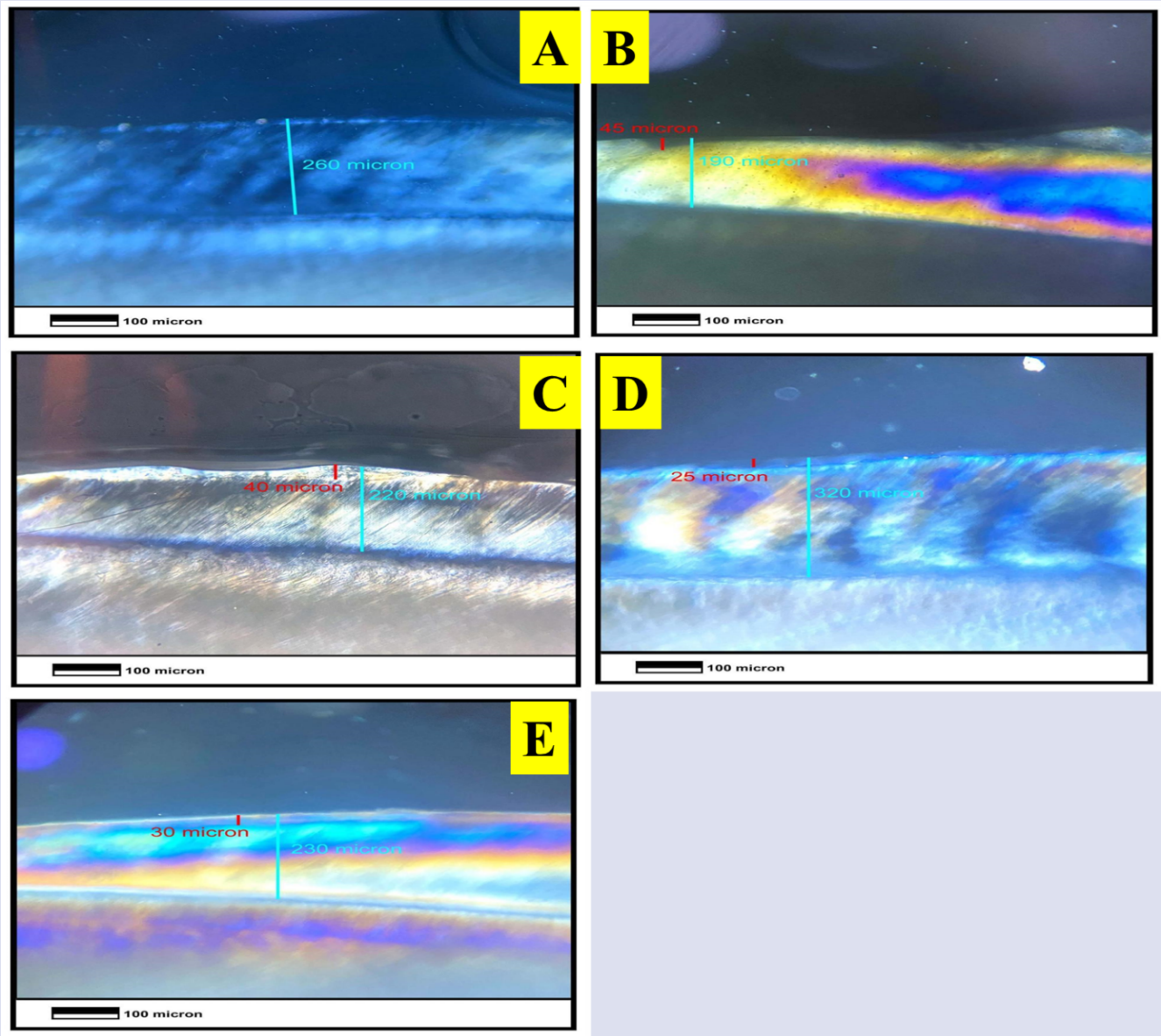

Figure 1: Longitudinal ground section of the enamel surface of the primary anterior tooth specimen. (A) baseline level, the turquoise line represents the full thickness of enamel at baseline. (B) after being exposed to Iron[plus] syrup. The turquoise line represents the full thickness of enamel whereas the red one represents the amount of erosion. (C) after being treated with ProShield[R] varnish. The turquoise line represents the full thickness of enamel whereas the red one represents the amount of remineralization. (D) after being treated with Zamzam water. The turquoise line represents the full thickness of enamel whereas the red one represents the amount of remineralization (E) after being treated with Zamzam water and ProShield[R] varnish. The turquoise line represents the full thickness of enamel whereas the red line represents the amount of remineralization.

plus Zamzam Water followed by the group treated with Varnish alone then the group treated with Zamzam Water alone. ANOVA test demonstrated the difference among the tested groups at baseline and after treatment cycle and it was noticed that at baseline, no significant differences were found among the tested groups, while after exposure of samples to the treatment cycles, high significant differences were occurred among tested groups

Dhillon *et al.* (2020) concluded that the greater microhardness mean values after used of fluoride varnish may be because it aids in the formation of long-lasting intraoral fluoride reservoirs and because of its ability of forming fluorohydroxyapatite to enhance the remineralization of incipient demineralized lesions. Second, the varnish delivery system allows ion prolonged contact between fluoride and dental hard tissues, therefore providing ideal conditions for their interaction and at the same time preventing the immediate loss or fluoride alter application.[38]

Finding of current study were in agreement with the findings of Molaasadolah *et al.*, (2017) who utilized fluoride varnishes and concluded that there was a significant increase in tooth enamel microhardness after their application.[39] Al-Rawi *et al.*, (2009) pointed to the fact that 0.05% NaF was superior in its effect regarding remineralization compared to Zamzam Water and this is confirming the results in this study.[23]

Zamzam Water group as a second priority that came after Varnish in terms of remineralization to prevent enamel erosion in primary dentition and this is evident in the mean values which were remarkable since Zamzam water is multi-element water40. Calcium, phosphate and fluoride are components of interest. In addition, it is rich in bicarbonate. [26] The incorporation of Zamzam water elements (fluoride, magnesium, calcium) in appetite crystals helps in increasing acid dissolution resistance as the presence of fluoride component

in Zamzam water may be responsible for the chemical reaction between Zamzam water constituents and appetite crystals.[41] Also, Alawiyah *et al.* (2021) estimated that Zamzam water has high buffering capacity because it has high bicarbonate concentration which the main buffer component, and also high pH value (average pH of 8) which may be a major role on increasing the microhardness. In the oral cavity, the critical pH has been set at 5.5, Zamzam water had a pH higher than 5.5. In current study, the pH value for Zamzam water was 7.2 that play a role on increasing of the microhardness of teeth surface, suggesting that Zamzam water could be introduced as a part of the prevention of dental erosion in the primary dentition.[26]

Regarding microhardness man values of combination treatment of (Zamzam Water and Varnish) in the current study showed the highest levels of remineralization process compared to other two groups this 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 destructed crystals.[25]

The present study also explained the percentage of loss of surface microhardness for all study groups that had found. Thus, the result exhibited that, the percentage of loss of microhardness for fluoride plus zamzam water group was less than fluoride varnish group, Zamzam water group and the control (deionized water) group. Also, noticed that the percentage of loss of microhardness for fluoride varnish group was less than that of the Zamzam water group, hence both of the fluoride plus zamzam water group could prevent further loss of mineral during PH cycle (drug cycle) of enamel erosion, however providing additional minerals to protect the enamel of primary teeth. This result agreed with Kufiyah *et al.* (2021) who concluded that fluoride varnish more effectively prevent the dental erosion of the entire primary dentition in comparison with Zamzam group.[33]

The morphological results of Polarized Light Microscope provided evidence that the studied medicine could potentially erodes primary tooth enamel after successive immersion cycle. The Iron[plus] evaluated medicine has acids in its composition, which results in a low pH. This result in agreement with Kulkarni *et al.* (2016) which reported that as substance pH decreases, the potential of enamel erosion increases. Nevertheless, the erosive potential of a substance is not exclusively dependent on pH value and acid type but also titratable acidity, calcium chelation properties, mineral content, and adhesion to the dental surface.[14]

Also, the result of current study shows that enamel sample that was treated with Zamzam water or ProShield[R] varnish have a capacity to return lost minerals (remineralization). This because both contain fluoride. On theater hand, many experts suggest that certain individualities make Zamzam water better, such as a higher level of calcium and phosphate and fluoride, incorporation of Zamzam water elements (F, P and Ca^{+2}) in the appetite crystals enhancing the repair of enamel layer and the resistance to acid dissolution.[33,41] After application, fluoride on the enamel surface increases. This occurrence was probably related to the formation of CaF2-like materials on the enamel surface.[42] act reducing or slowing the contact between the acid and the underlying enamel as seem to act as a physical barrier, preventing contact between the acid and enamel or as a mineral reservoir, to buffer the acids, and increase remineralization.

The results of current study showed that when use both Zamzam water and ProShield[R] varnish, they succeeded to have a more condensed enamel similar to its original enamel suggesting that both Zamzam water and ProShield[R] varnish had a synergistic effect towards remineralization in an effective manner, the mixture therapy type had succeeded to penetrate deeply on jeopardized enamel. This result due the double action of ProShield[R] varnish plus Zamzam water which appeared to be the most effective in protecting enamel erosion when combined together compared to other groups. The PLM findings of this present study, come in line with the outcomes of surface microhardness as the loss of enamel surface and the porosity seen in the PLM images of the enamel surface exposed to this pediatric syrup are in accordance with the reduction in surface microhardness mean values. Besides, that the various remineralizing agents used in this study rebuild the lost surface and consequently restore the hardness of teeth.

CONCLUSION

Iron[plus] medicinal syrup possess a high erosive potential to enamel tissue due to the existence of acid components in their chemical formulation and thus reduction in microhardness of primary tooth enamel was apparent. In contrast, when treating the samples with ProShield[R] varnish, Zamzam water and their combination remedies, the results of vickers microhardness and polarized light microscope confirmed their remineralizing capability to protect against acidic dental erosion.

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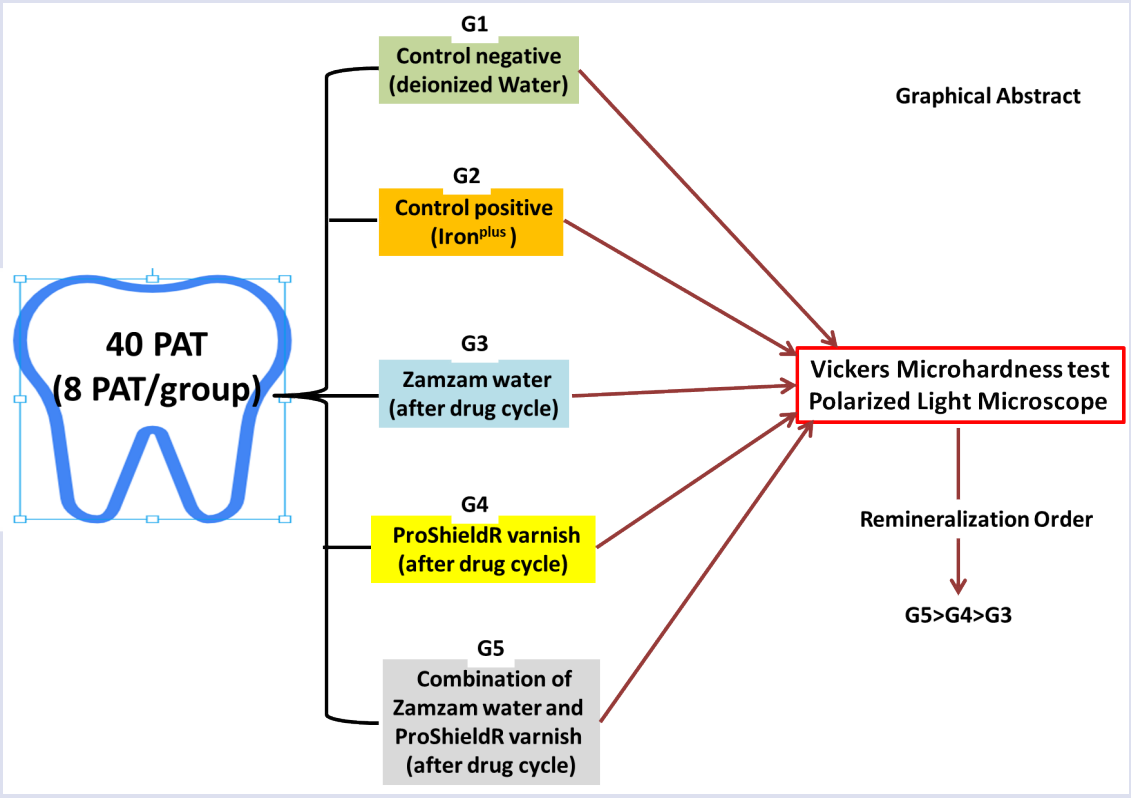
GRAPHICAL ABSTRACT



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