

## COMPARISON BETWEEN SOLUTION AND GEL FORMS OF THEOBROMINE AND SODIUM FLUORIDE IN REMINERALIZATION OF THE DEMINERALIZED HUMAN ENAMEL (SEM AND EDXA STUDY)

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### **ABSTRACT**

**Introduction:** Enamel demineralization occurs by acid attack during dental caries. The initial caries could be arrested by enhancement of remineralization via topical fluoride application. Although fluoride is effective in enamel protection, the safety of its use is a matter of controversy which necessitated the search for new alternatives. Theobromine is one of the promising materials in caries prevention which is safer than fluoride. The effect of the remineralizing material consistency “gel or solution” is essential to be evaluated to reach the optimum protocol.

**Purpose:** To compare between the gel and solution forms of both theobromine and fluoride in the remineralization potential of the demineralized human enamel.

**Materials & Methods:** Thirty longitudinal halves of human mandibular premolars were equally divided into 6 groups: control (C), demineralization (D) subjected to demineralizing solution then 5 days PH cycle. The remaining 4 groups were similar to demineralization group with addition of treatment material “theobromine solution (T1), theobromine gel (T2), fluoride solution (F1) and fluoride gel (F2)”. The samples were investigated by scanning electron microscope (SEM) and energy dispersive x ray analysis (EDXA).

**Results:** The enamel of demineralization group was porous with erosive changes exposing the subsurface enamel rods with severe rod core defects. Theobromine solution and fluoride gel groups showed improvement of the enamel surface. Fluoride solution group showed more observable enamel defects while the least favorable enamel topography was observed in the theobromine gel group. EDXA revealed that the calcium phosphorus ratio displayed a descending order: (C > T1 > F2 > F1 > T2 > D).

**Conclusion:** Theobromine solution and fluoride gel are more effective remineralizing agents. Fluoride solution and theobromine gel have less remineralizing potential.

**KEYWORDS:** Theobromine, fluoride, remineralization, SEM, EDXA.

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## INTRODUCTION

Enamel is regularly subjected to demineralization and remineralization under different oral circumstances. Any interruption in the balance between demineralization and remineralization leads to caries<sup>(1,2)</sup>. However, dental caries in its early stage of formation can be remineralized<sup>(3)</sup>, fluoride therapy is one of the most popular and effective methods of caries prevention either in systemic mode (tablets, salt, milk, or water)<sup>(4)</sup> or topical fluoride application<sup>(5,6)</sup>.

The presence of fluoride in saliva and plaque in case of a carious lesion can inhibit the dissolution of enamel crystals therefore enhancing remineralization<sup>(7)</sup>. The anticariogenic action of fluoride is achieved through inhibition of demineralization and enhancement of remineralization<sup>(8)</sup>. Calcium fluoride, calcium hydroxide, and fluorapatite are produced after fluoridation. These fluoride compounds protect enamel surface and act as fluoride reservoirs<sup>(9,10)</sup>.

One of the determining factors of the fluoride effectiveness is the fluoride product consistency. Topical fluoride application to prevent caries includes fluoride containing rinses, toothpastes, gels, solutions, and varnishes<sup>(11)</sup>. Fluoride gel and dentifrices were recommended by many authors<sup>(12-14)</sup>. On the other hand, fluoride solutions were reported to be effective remineralizing agents<sup>(15,16)</sup>. Moreover, fluoride varnish also has been widely used in high-caries-risk individuals, although there is still some debate about whether this vehicle is best for prevention or for repair of non-cavitated lesions<sup>(17-20)</sup>.

Although fluoride could be considered a favorable alternative in caries prevention, excessive fluoride intake can cause fluorosis, tooth damage, brittle bones as well as decreased children intelligence, early aging, spontaneous abortion and digestive tract irritation<sup>(21)</sup>. Moreover, the ability of fluoride to promote remineralization is limited by the availability of calcium and phosphate in

saliva<sup>(22)</sup>. Thus, fluoride therapy alone is insufficient to control the caries process in high caries-risk individuals<sup>(23)</sup>. Because of these characteristics of fluoride, many alternatives as well as adjunctive products were presented in the last decades including casein phosphopeptide with amorphous calcium phosphate<sup>(24)</sup>, Tri-Calcium Phosphate added to fluoride<sup>(25)</sup>, glass Ionomer Cements<sup>(26)</sup> and iron<sup>(27)</sup>.

One of the promising materials for caries prevention is theobromine (dimethyl xanthine) which is a white crystal powder that can be differentiated from caffeine by one methyl group. It is an alkaloid compound found in chocolate. It has been reported that theobromine can be used to prevent enamel demineralization<sup>(28)</sup> and to enhance the remineralization potential<sup>(29)</sup>. The enamel surface microhardness was improved after theobromine application which could be an indication for the protective effects of theobromine on enamel<sup>(30,31)</sup>. The gel form of theobromine was investigated, it was reported that theobromine-containing toothpastes have a potential in occluding dentin tubules<sup>(32)</sup>.

The comparison between theobromine and fluoride was conducted by many authors and their results were relatively inconsistent. In a study done by **Amaechi et al., 2013**, it has been reported that theobromine may be a possible alternative to fluoride additives in dentifrices. Since theobromine, at a molar level 71 times less than that of fluoride showed remineralization ability on enamel lesions comparable to that of fluoride<sup>(29)</sup>. However **Nakamoto et al., 2016**<sup>(34)</sup> and **Mahardhika et al., 2017**<sup>(34)</sup> reported that theobromine and fluoride have similar effects on enamel surface and remineralization ability. Theobromine was more favorable as it is safer because of its low toxicity when compared to fluoride<sup>(33)</sup>. The comprehensive comparison between different forms of theobromine and fluoride in remineralization ability of the demineralized enamel is essential to clarify the optimum caries prevention protocol.

## MATERIALS AND METHODS

### Sample selection and preparation

Twenty sound human mandibular first premolars were used in the present study. Teeth were examined to exclude those with enamel abrasions, erosions or decay on the buccal surfaces. Each tooth was sectioned longitudinally using diamond disk with copious water coolant to avoid heat generation. Thirty halves of the sectioned teeth were further selected and divided into 6 equal groups (5 samples each):

- Control group “C”: Samples were stored in distilled water during experiment period.
- The remaining 25 samples were subjected to **demineralization protocol** to initiate caries like lesion guided by previous studies with minor modification<sup>(35, 36)</sup>; samples were immersed 4 days in demineralizing solution [2.2 millimole (mM) Calcium Chloride, 50 mM Acetic Acid and 2.2 mM Sodium Dihydrogen Phosphate]. The PH of the solution was adjusted around 4.5 by the addition of Potassium Hydroxide.
- After initiation of caries like lesions, the samples were subjected to PH cycle for 5 days and subdivided into 5 equal groups according to the treatment applied during the PH cycle. Demineralization group “D”: no treatment applied. Groups “T1 and T2”: treated with

200mg/liter theobromine solution and gel respectively. Groups “F1 and F2”: treated with 200mg/liter sodium fluoride solution and gel respectively (table 1).

- **The PH cycle** similar to that proposed by *Argenta et al., 2003*<sup>(37)</sup>:
  1. Application of treatment material (fluoride or theobromine) for a period  $\approx$  3 minutes.
  2. Immersion in the demineralizing solution for 3 hours.
  3. Application of the treatment material (fluoride or theobromine)  $\approx$  3 minutes.
  4. Immersion in the remineralizing solution till the beginning of the next cycle. Remineralizing solution is composed of (1.5 mM calcium chloride, 0.9 mM sodium phosphate) with PH adjusted to 7 by addition of potassium hydroxide.
- The theobromine (3,7-Dimethylxanthine) and sodium fluoride were supplied in powder form. The solution of each material was prepared by dissolution of the specified concentration in deionized water. While the gel was obtained by addition of hydroxy-ethyl cellulose powder to the solution (60 gm/liter).

SEM examination & Energy Dispersive X ray Analysis (EDXA):

TABLE (1): Summary of the studied groups

Group	Group name	Demineralization	PH cycle	Treatment	Sample number
C	Control	No	No	No	5
D	Demineralization	Yes	Yes	No	5
T1	Theobromine solution	Yes	Yes	Theobromine solution	5
T2	Theobromine gel	Yes	Yes	Theobromine gel	5
F1	Fluoride solution	Yes	Yes	Fluoride solution	5
F2	Fluoride gel	Yes	Yes	Fluoride gel	5

The middle third of the buccal surface enamel of each sample was examined under the magnification of (500, 1000 and 2000) to evaluate the surface characteristics of enamel. Quantitative analysis of surface mineral content of enamel samples using (EDXA) was performed to chemically analyze and measure calcium (Ca) and phosphorus (P) atomic % of each enamel sample under magnification (200x) to cover wide fixed area. Each enamel sample was placed under vacuum and excited to a higher energy state with an electron beam. As the electrons of each element falls back down to its original energy state, it emits x-ray energy at different specified wave length.

The data of calcium (Ca) and phosphorus (P) percentage were tabulated and the Ca/P ratio was calculated as an indication of the mineral changes during demineralization and remineralization. The mean values of Ca/P ratio were calculated for each group. Results were tabulated and statistically analyzed using one-way ANOVA test to compare between groups followed by Post Hoc test (pairwise comparison with Bonferroni adjustment of P value) to compare between each 2 groups.

## RESULTS

### Scanning Electron Microscopic results:

Examination of the middle third of buccal surface enamel of the control group revealed regular relatively smooth enamel surface free of

erosive lesions or porosity. The flat surface didn't show evidence of the characteristic rod cross section, indicating that this flat surface is the intact outer rodless layer. Rarely, minute few depressions were detected (fig. 1). The demineralization group displayed scattered porosity almost all over the surface with separate areas of erosive changes. The outer rodless layer was removed exposing the enamel rods with fish-scale pattern. Many of the exposed rods presented severe defects in the rod core. Occasionally some defects involved groups of neighboring rods forming larger defect (fig. 2).

The enamel surface of theobromine solution group demonstrated smooth enamel surface in most of regions. While the rod ends were exposed in some areas with circular concavities. Occasionally, there were variable sized deep pits (fig. 3). On the other hand, theobromine gel group had rough enamel surface with apparent surface defects. Wide irregular deep depressions were clearly seen scattered on the enamel surface (fig. 4). Sodium fluoride solution group showed sharp edged depressions with variable depth. Interestingly, aggregations of globular masses were detected over the enamel surface. Many areas of the affected enamel revealed erosive non-pitted lesions with deeply dark appearance rather than porous defects (fig. 5). Sodium fluoride gel group displayed smooth enamel surface with no observed pits or exposed enamel rods. However, several regions of enamel showed erosive defects with variable sizes which lead to appearance of rough surfaced enamel areas (fig. 6).

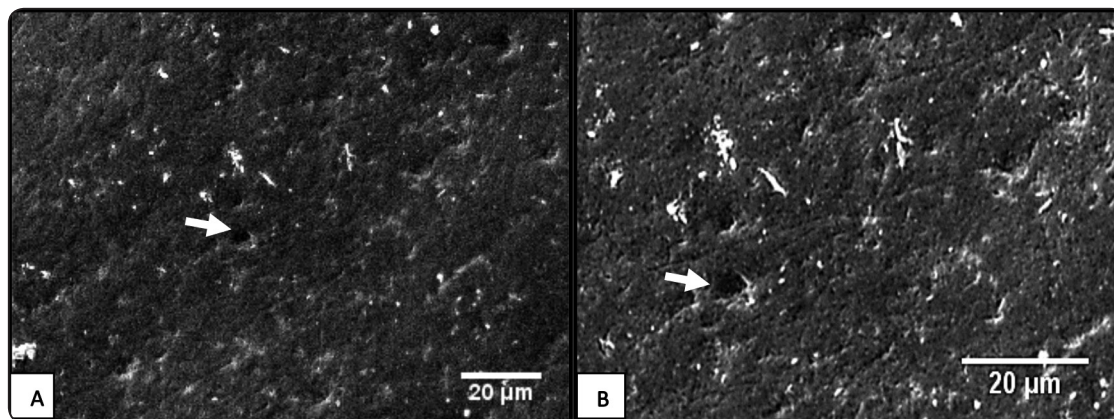


Fig. (1) Scanning electron micrographs of control group showing relatively smooth enamel surface with occasional minute few depressions (arrows). [Magnification: A (x500) B (x1000)].



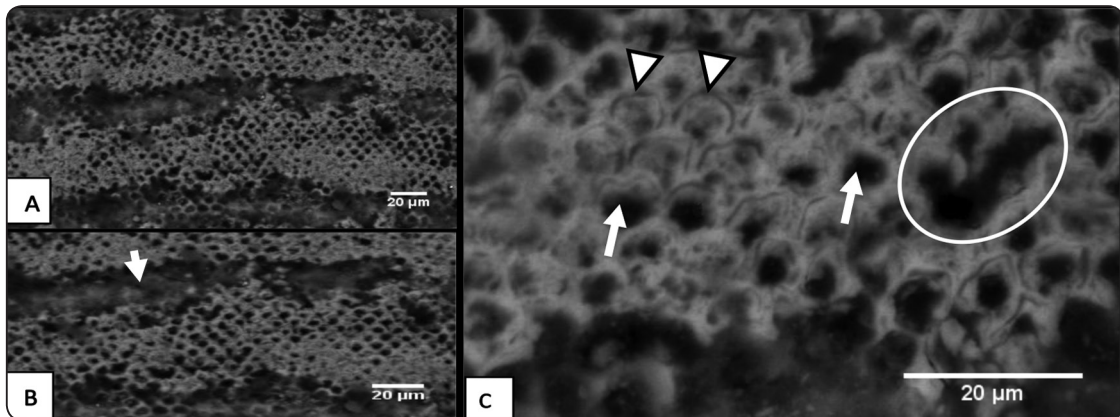


Fig. (2) Demineralization group. (A): Porous enamel surface with erosive changes (arrow). (B): Removed outer rodless layer exposing the rods and their sheath with fish-scale pattern (arrow heads), many rods revealed severe defects in the rod core (arrows). Occasionally some defects involved groups of rods forming larger defect (oval) [Magnification: A (x500) B (x1000) C (2000)].

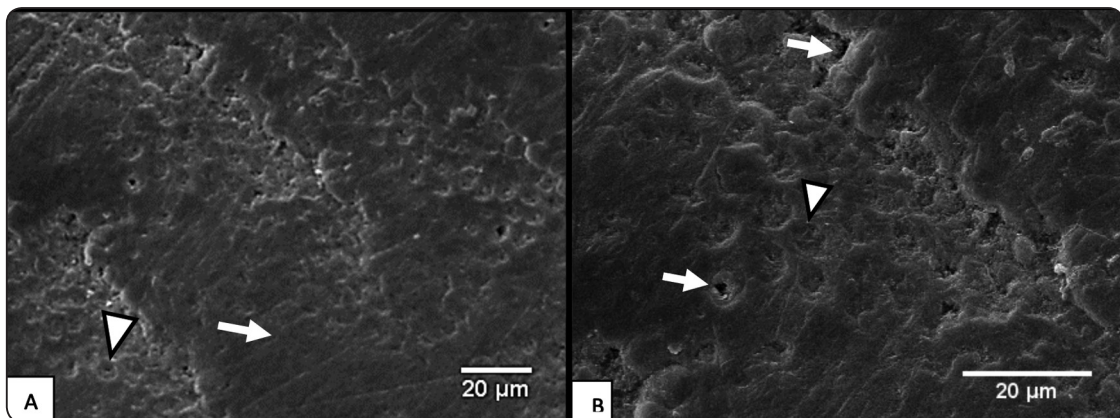


Fig. (3) Scanning electron micrographs of theobromine solution group. (A): Smooth enamel in many regions (arrow) while the rod ends are exposed in other areas (arrow head). (B): The enamel rod end displayed shallow rounded dipping (arrow head) and occasionally variable sized deeper pores are observed (arrows) [Magnification: A (x500) B (x1000)].

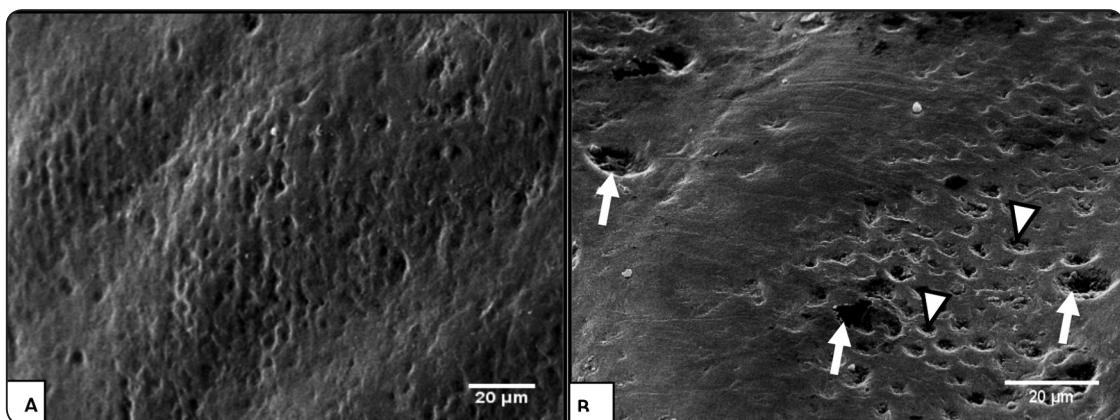


Fig. (4) Scanning electron micrographs of theobromine gel group. (A): rough enamel surface. (B): The regular circular pits on enamel surface (arrow heads) and wider irregular depressions scattered on the enamel surface (arrows) [Magnification: A (x500) B (x1000)].

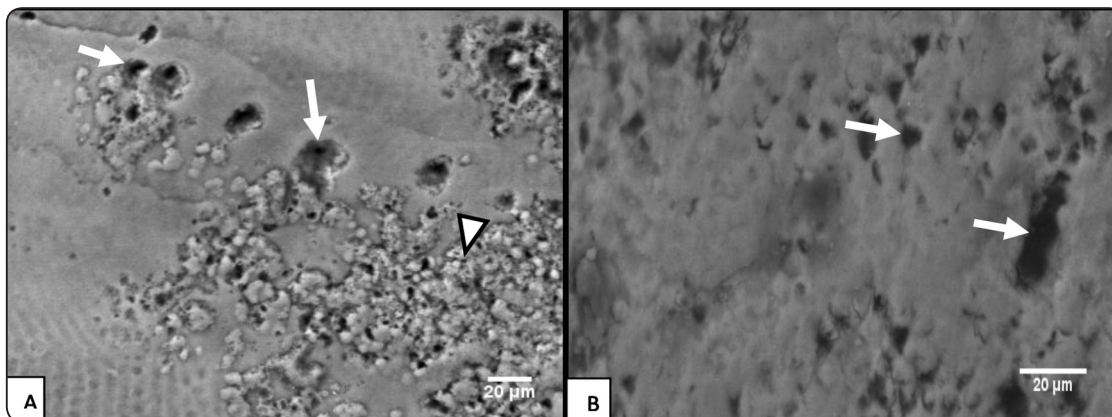


Fig. (5) Scanning electron micrographs of sodium fluoride solution group. (A): several depressions with variable depth (arrows) in addition to aggregation of globular masses over the enamel surface (arrow head). (B): Some areas of the affected enamel revealed erosive non-pitted lesions with deeply dark appearance (arrows) rather than porous defects [Magnification: A (x500) B (x1000)].

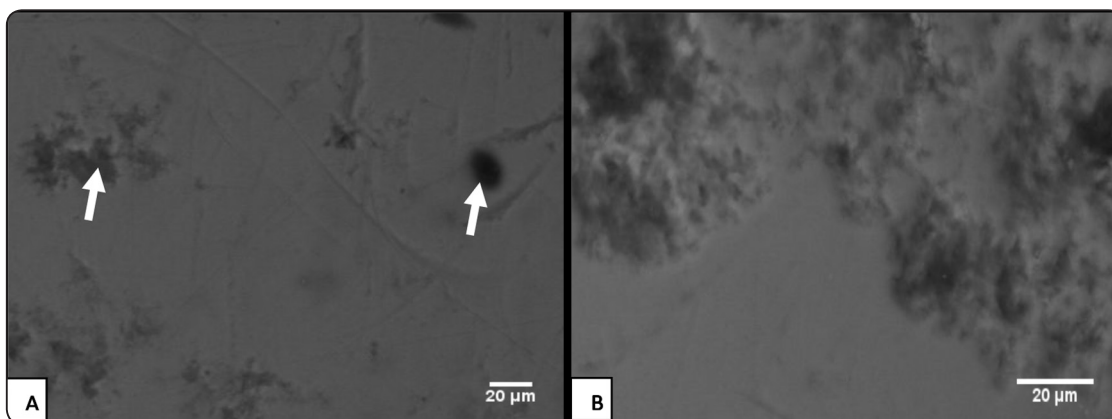


Fig. (6) Scanning electron micrographs of sodium fluoride gel group. (A): Smooth enamel surface with no observed pits or exposed enamel rods. Variable sized and colored erosive lesions are detected (arrows). (B): Higher magnification showing the minor surface irregularities with almost no observable porous areas in enamel surface [Magnification: A (x500) B (x1000)].

**Energy Dispersive X ray Analysis results:**

The average percentages of the calcium and phosphorus were measured and then the Ca/P ratio was calculated for each sample. The mean and standard deviation were calculated. The control group showed the highest mean value while the least mean value was of the demineralization group. The order of the 6 groups in a descending manner is: control, theobromine solution, sodium fluoride gel, sodium fluoride solution, theobromine gel and finally demineralization group (table 2 and fig. 7). ANOVA single factor test revealed nonsignificant

difference between the 6 groups (P value > 0.05). The comparison between each 2 groups showed significant difference between the demineralization group and the fluoride gel group (P value < 0.05) while the other comparisons were statistically nonsignificant.

**Table (2):** The mean values and standard deviation of the Ca/P ratio in all groups.

	C	D	T1	T2	F1	F2
<b>Mean</b>	2.04	1.45	1.98	1.56	1.59	1.64
<b>Standard deviation</b>	0.84	0.00	0.42	0.25	0.02	0.05

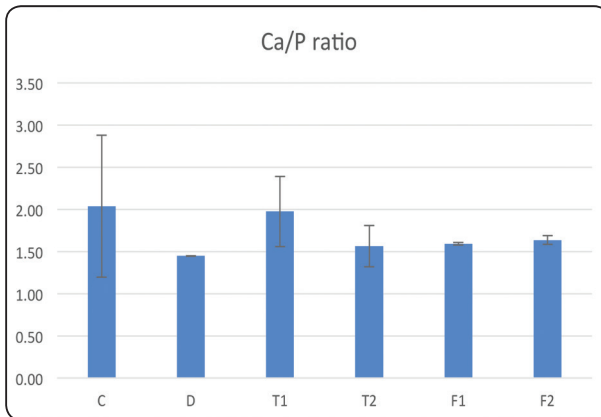


Fig. (7) Bar chart showing the mean values of the Ca/P ratio in all groups.

## DISCUSSION

Caries prevention is an important aim in the dental field. The present work focused on the remineralization as an effective tool for reversing an incipient caries. Although the enamel remineralization potential and its role in caries arresting are widely discussed in the literature<sup>(38)</sup>. There is controversy about the categorization of the remineralizing materials as well as the techniques for application of these materials. We selected two remineralizing materials; the first sodium fluoride which is considered for long time the most effective method of caries prevention<sup>(39)</sup>. The second is theobromine, a promising caries preventive material comparable to fluoride but safer than fluoride as theobromine is non-toxic<sup>(30)</sup>. Moreover, the present work shed a light also on the effect of the consistency on the remineralization potential using gel and solution forms for each of the studied materials.

The initiation of enamel caries like lesions in our work was performed by chemical invitro models due to their simplicity, low cost, and experimental stability<sup>(40)</sup>. The demineralizing solution used in the present work was mainly of acetic acid as it could cause detectable lesion formation, even at pH 5.0 or higher<sup>(41)</sup>. In the present work, after initiation of caries like lesions we used the PH cycling models to mimic the in vivo periodic alternation of PH<sup>(35)</sup>.

The use of PH cycles could represent the oral environment more closely than in traditional experimental designs. Furthermore, the efficient scientific control of the experiment lead to lower variability<sup>(42)</sup>.

We observed erosive areas in the enamel in the samples subjected to the acidic demineralizing solution. Similar organic acids as lactic acid and citric acid were also reported in previous studies to predispose areas of enamel surface dissolution as well as erosion foci<sup>(43,44)</sup>. Moreover, we presented in our results porous enamel surface after demineralization. This coincides with *Karlinsey et al 2012* who reported that the acid application dissolves calcium and phosphate ions which causes gapping between crystals which, in turn, lead to enamel porosity<sup>(44)</sup>. The EDXA in our results supported the SEM results as the demineralization group showed decreased Ca/P ratio indicating demineralization process chemically. This could be explained by the drop of PH below a certain level, thus, enamel hydroxyapatite dissolve, and demineralization occurs<sup>(40)</sup>.

In the present work, Our results revealed an improvement in the enamel surface characteristics in all the treated groups with different degrees. Regarding the porosity, we observed that generally fluoride groups (particularly fluoride gel) were more favorable than theobromine groups. This might be explained by the mutual effect of fluoride in both inhibition of demineralization by protecting the crystals from dissolution<sup>(45)</sup> as well as enhancement of remineralization by adsorption to the surface of the demineralized crystals and attraction of calcium ions<sup>(46)</sup>. Our results also coincide with *Carrillo et al 2018* who concluded that fluoride is the most effective treatment for remineralization of non-cavitated lesions.

In the present study, EDXA revealed more improved Ca/P ratio in the theobromine solution group than in the fluoride groups. While the theobromine gel group was of the least value, but these results were statistically nonsignificant. On the contrary, *Kargul et al 2012* reported a superior effect



of fluoride in increasing the surface microhardness of the demineralized enamel more than theobromine<sup>(30)</sup>. The controversy might be due to the difference in the fluoride form as the authors used acidulated phosphate fluoride while in our work, we used sodium fluoride.

The improvement in surface chemical composition of enamel with the theobromine solution group agreed with *Nakamoto et al 2016* who concluded that theobromine caused formation of larger HAP crystallites in vitro<sup>(33)</sup>. Moreover, *Sadeghpour et al 2007* stated that theobromine causes calcium and phosphate to merge into a crystal unit that is four times bigger than hydroxylapatite<sup>(29)</sup>. Our results revealed that although theobromine solution caused increase in the Ca/P ratio but didn't reach the level of the control group. This coincides with *Irawan et al 2017* who concluded that theobromine increased enamel surface hardness after demineralization but did not restore it to its initial hardness<sup>(48)</sup>.

In the present study, SEM and EDXA of theobromine groups revealed inferior enamel characteristics in comparison to the other groups particularly the theobromine solution group. The effective action of theobromine solution was reported in the literature especially in apatite-forming medium like the remineralizing solution used in the present work<sup>(29)</sup>. However, our finding regarding the theobromine gel didn't coincide with *Syafira et al 2012* who considered that theobromine gel a potential dental caries prevention material due to its effect in improving the enamel surface microhardness<sup>(49)</sup>. However, the authors focused on the surface microhardness, a different parameter from our work. Furthermore, *Syafira et al 2012* studied theobromine gel of different concentrations but they did not compare between gel and solution. Moreover, the authors didn't mention that theobromine gel repaired the enamel porosity; they claimed that enamel pores increase penetration of theobromine gel on the enamel surface, which causes elevation of apatite crystals which, in turn, hardened the enamel.

## CONCLUSIONS

Within the limits of the present study, we concluded that all the tested materials had a remineralization potential. Theobromine solution and fluoride gel are slightly more potent remineralizing agents than the other studied forms. In vivo studies are necessary to confirm this hypothesis.

## REFERENCES

- 1- O'Mullane DM. Introduction and rationale for the use of fluoride for caries prevention. *Int Dent J*.1994; 44:257-261.
- 2- Zero DT. Dental caries process. *The Dental Clinics of North America Cariology*.1999; 43:635-664.
- 3- Espelid I. Caries preventive effect of fluoride in milk, salt, and tablets: A literature review. *European Archives of Paediatric Dentistry*.2009; 10 (3), 149-156.
- 4- Featherstone JDB: Dental caries: a dynamic disease process. *Aust Dent J*. 2008; 53:286-291.
- 5- Thurnheera T and Belibasakis GN. Effect of sodium fluoride on oral biofilm microbiota and enamel demineralization. *Archives of oral biology*. 2018; 89:77-83.
- 6- Hamdan WA, Badri S and El Sayed A. The effect of fluoride varnish in preventing enamel demineralization around and under orthodontic bracket. *Int Orthod*. 2018; 16(1):1-11.
- 7- Shellis RP and Duckworth RM. Studies on the cariostatic mechanisms of fluoride. *Int Dental*.1994; 44: 263-273.
- 8- Wiegand A, Buchalla W and Attin T. Review on fluoride-releasing restorative materials—fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. *Dent. Mater*.2007; 23: 343-362.
- 9- Gerth HUV, Dammaschke T, Schäfer E and Zuchner H. A three layer structure model of fluoridated enamel containing CaF<sub>2</sub>, Ca(OH)<sub>2</sub> and FAp. *Dent. Mater*.2007; 23: 1521-1528.
- 10- Campillo M, Lacharaise PD, Reparaz JS, Goni AR and Valiente M. On the assessment of hydroxyapatite fluoridation by means of Raman scattering. *J. Chem. Phys*.2010; 132(24): 244501-244505.
- 11- Hicks J, Garcia-Godoy F and Flaitz C. Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). *J. Clin. Pediatr. Dent*.2004; 28: 203-214.



- 12- Nordstrom A and Birkhed D. Preventive effect of high-fluoride dentifrice (5,000 ppm) in caries-active adolescents: a 2-year clinical trial. *Caries Res.*2010; 44:323-331.
- 13- Villena RS, Tenuta LMA and Cury JA. Effect of APF Gel Application Time on Enamel Demineralization and Fluoride Uptake In Situ *Braz Dent J.* 2009; 20(1): 37-41.
- 14- Delbem ACB and Cury JA. Effect of application time of APF and NaF gels on microhardness and fluoride uptake of in vitro enamel caries. *Am J Dent.* 2002; 15:169-172.
- 15- Hirce JD, Sather AH and Chao EY. The effect of topical fluorides, after acid etching of enamel, on the bond strength of directly bonded orthodontic brackets. *Am J Orthod.* 1980; 78:444-452.
- 16- Leodigo GDR, Fernandes HO, Tonetto MR, Cristina Dupim Presoto CD, Bandeca MC and Firoozmand LM. Effect of Fluoride Solutions on the Shear Bond Strength of Orthodontic Brackets. *Braz Dent J.* 2012; 23(6): 698-702.
- 17- Autio-Gold JT and Courts F. Assessing the effect of fluoride varnish on early enamel carious lesions in the primary dentition. *J Am Dent Assoc.*2001; 132:1247-1253.
- 18- Bader JD, Shugars DA and Bonito AJ. A systematic review of selected caries prevention and management methods. *Community Dent Oral Epidemiol.* 2001; 29:399-411.
- 19- Du M, Cheng N, Tai B, Jiang H, Li J and Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. *Clin Oral Investig.* 2011; 16:463-468.
- 20- Ferreira JM, Aragao AK, Rosa AD, Sampaio FC and Menezes VA. Therapeutic effect of two fluoride varnishes on white spot lesions: a randomized clinical trial. *Braz Oral Res.* 2009; 23:446-451
- 21- Aravind A, Dhanya RS, Narayan A, Sam G, Adarsh VJ and Kiran M. Effect of fluoridated water on intelligence in 10-12-year-old school children. *Journal of International Society of Preventive and Community Dentistry.*2016; 6 237-242.
- 22- Mandel ID. Impact of saliva on dental caries. *Compend Suppl.*1989; 13:476-481.
- 23- Featherstone JD and Doméjean S. The Role of Remineralizing and Anticaries Agents in Caries Management. *Adv Dent Res.* 2012; 24(2): 28-31.
- 24 Cross KJ, Huq NL, Reynolds EC, Casein Phosphopeptides in oral health-chemistry and clinical applications. *Curr Pharm Des.* 2007; 13(8):793-800.
- 25- Karlinsey RL and Mackey AC. Solid-State Preparation and Dental Application of an Organically Modified Calcium Phosphate. *J Mater Sci.* 2009; 44:346-349.
- 26- Shimazu K, Ogata K and Karibe H. Caries-preventive effect of fissure sealant containing surface reaction-type pre-reacted glass ionomer filler and bonded by self-etching primer. *J Clin Pediatr Dent.* 2012; 36(4):343-347.
- 27- PintoAlves KMR, SimõesFranco K, Sasaki KT, Buzalaf MAR, BotazzoDelbem AC. Effect of iron on enamel demineralization and remineralization in vitro. *Archives of Oral Biology.* 2011; 56(11):1192-1198.
- 28- Sadeghpour A. A neural network analysis of theobromine vs. fluoride on the enamel surface of human teeth. An experimental case study with strong implications for the production of a new line of revolutionary and natural non-fluoride based dentifrices. *Dissertation Abstracts International.* 2007; 68(7) suppl.B: 150.
- 29- Amaechi BT, Porteous N, Ramalingam K, Mensinkai PK ,Cahuana Vasquez RA , Sadeghpour A , Nakamoto T . Remineralization of Artificial Enamel Lesions by Theobromine. *Caries Res.* 2013; 47:399-405.
- 30- Kargul B, Özcan M, Peker S and Falster AU. Evaluation of human enamel surfaces treated with theobromine: a pilot study. *Oral health & preventive dentistry.* 2012; 10(3):275-282.
- 31- Syafira G, Permatasari R and Wardani N. Theobromine Effects on Enamel Surface Microhardness: In Vitro. *Journal of Dentistry Indonesia.* 2012; 19(2): 32-36.
- 32- Amaechi BT, Mathews SM and Mensinkai PK. Effect of theobromine-containing toothpaste on dentin tubule occlusion in situ. *Clin Oral Investig.* 2015; 19(1):109-116.
- 33- Nakamoto T, Falster A U and Simmons W B. Theobromine: A Safe and Effective Alternative for Fluoride in Dentifrices *Journal of Caffeine Research.* 2016; 6: 1-9.
- 34- Mahardhika A, Noerdin A and Eriwati Y K .The effects of brushing on human enamel surface roughness after NaF gel and theobromine gel exposure. *Journal of Physics Conference Series.* 2017; 884(1):012007
- 35- Tencate JM and Duijsters PE. Altering Demineralization and Remineralization of Artificial Enamel Lesions. *Caries Res.J.* 1982; 16 (3): 201-210.
- 36- Tschoppe P, Zandim DL, Martus P and Kielbass AM. Enamel and Dentin Remineralization by Nanohydroxyapatite toothpastes. *J.Dent.* 2011; 39:430-437.

- 37- Argenta, RMO, Tabchoury, CPM and Cury JA. (). A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. *Braz dent. J.* 2003; 17(3):241-246.
- 38- Abou Neel EA, Bozec L, Perez RA, Kim H-W and Knowles JC. Nanotechnology in dentistry: prevention, diagnosis, and therapy. *Intell Surf.* 2015; 10:6371-6394.
- 39- Jensen O, Gabre P, Sköld UM and Birkhed D. Is the use of fluoride toothpaste optimal? Knowledge, attitudes and behaviour concerning fluoride toothpaste and toothbrushing in different age groups in Sweden. *Community Dent Oral Epidemiol.* 2012;40(2):175-184.
- 40- Yiru OY, Shuping IZ, Lei MM, Chin-Man EL and Chun-Hung C. A Review of the Common Models Used in Mechanistic Studies on Demineralization-Remineralization for Cariology. *Research. Dent. J.* 2017; 5 (20): 1-8.
- 41- Featherstone JDB and Rodgers BE. Effect of Acetic, Lactic and other Organic Acids on the Formation of Artificial Carious Lesions. *Caries Res.* 1981;15:377-385.
- 42- Buzalaf MAR, Hannas AR, Magalhaes AC, Rios D, Honorio HM and Delbem ACB. Ph-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: Strengths and limitations. *J. Appl. Oral Sci.* 2010; 18: 316-334.
- 43- Neves AA, Primo LG and Ramos PR. Microscopic investigation of artificially demineralized surface enamel exposed to controlled intra-oral periods. *Australian Dental Journal.* 2003; 48(4):248-25.
- 44- Karlinsey RL, Mackey AC, Blanken DD and Schwandt CS. Remineralization of eroded enamel lesions by simulated saliva in vitro. *Open Dent. J.* 2012; 6: 170-176.
- 45- Featherstone JDB. Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol.* 1999; 27: 31-40.
- 46- Ten Cate JM and Featherstone JDB. Mechanistic aspects of the interactions between fluoride and dental enamel. *Crit Rev Oral Biol.* 1991; 2:283-296.
- 47- Carrillo EL, Rojas NL, Luckie RAM, Bermeo NLR, Fabila MMG, Santillana RD and Solís CEM. The Effects of Remineralization via Fluoride Versus Low-Level Laser IR810 and Fluoride Agents on the Mineralization and Microhardness of Bovine Dental Enamel. *Appl. Sci.* 2018, 8(1), 78.
- 48- Irawan MIP, Noerdin A and Eriwati YK. The effect of theobromine 200 mg/l topical gel exposure duration against surface enamel hardness resistance from 1% citric acid. *Journal of Physics: Conf. Series.* 2017, 884: 012009.
- 49- Syafira G, Permatasari R and Wardani N. Theobromine Effects on Enamel Surface Microhardness: In Vitro. *Journal of Dentistry Indonesia.* 2012; 19 (2): 32-36.