Effect of Novel Theobromine Varnish Versus Fluoride Varnish on the Remineralization, Microhardness and Color Change of Enamel in Extracted Carious Primary Molars: In-vitro Study

Radwa M. Galal[1], Mahmoud M. Hamdy[2], Nada M. Wassef [3]

[1] Post Graduate Student, Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University, Egypt
[1]Teaching Assistant, Pediatric Dentistry Department, Faculty of Dentistry, MSA University, Cairo, Egypt. Email: radwa.radwan@dentistry.cu.edu.eg
[2] Professor, Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University, Egypt.
[3] Associate Professor, Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University.
nada.wassef@dentistry.cu.edu.eg

Corresponding Authors: Radwa M. Galal
radwa.radwan@dentistry.cu.edu.eg

Abstract

Aim**:** Assessment of the effect of novel theobromine varnish on the remineralization, microhardness and color change of enamel compared to fluoride varnish in extracted carious primary molars.

Methods: Ten extracted primary molars were collected from the pediatric dentistry clinic, MSA University. The teeth were sectioned mesiodistally and divided into 2 equal groups (10 specimens each). The specimens were embedded into acrylic resin, subjected to demineralization followed by pH cycling. Test group (A) was coated with theobromine varnish while control group (B) was coated with 3M Clinpro fluoride vanish. The enamel was measured using SEM/EDXA, measured for microhardness using Vickers' microhardness tester and color change using Vita EasyShade V before, after demineralization and after remineralization.

Results: There was no significant difference in calcium and phosphorous concentrations in both groups. Test group showed higher values with significant difference in microhardness. Regarding color change, both the test and control groups showed no significant difference.

Conclusion: Theobromine varnish can be used as a safer alternative to fluoride varnish.

Keywords: Theobromine, fluoride, remineralization, microhardness, color change, artificially induced caries, primary molars

DOI: 10.48047/ecb/2023.12.8.767

I. INTRODUCTION

Dental caries is the most prevalent chronic disease worldwide. It is an infectious disease characterized by a multifactorial etiology that leads to the destruction of dental hard tissues. The implementation of preventive measures, the need of investing in education for the correct maintenance measures of oral health, associated with preventive and continuous medical and dental care, are key to the awareness of populations of its existence and to the decline of its prevalence **(Veiga *et al.*, 2016).**

White spot lesions (WSLs) are defined as enamel surface and subsurface demineralization, without cavitation. These manifestations represent the first clinical observation of the progression of dental caries, with the possibility of being reversed. WSLs develop as a result of prolonged plaque accumulation on the affected surface, commonly due to inadequate oral hygiene. With the maintenance of these conditions, acids diffuse into the enamel and begin the demineralization of subsurface enamel. If the demineralization process is not stopped, the intact enamel surface eventually collapses and cavitates **(Paula *et al.*, 2017)**.

The treatment of WSLs usually includes non-invasive approaches, such as remineralization, which is the first line of treatment, minimally invasive approaches such as resin infiltration, or invasive approaches such as micro-abrasion and composite restorations**(Hammad *et al*., 2020)**.

The commonly used agents for the treatment of WSLs are topical fluorides. These include fluoride toothpastes, fluoride varnishes, fluoride mouth rinses and fluoride gels **(Singh *et al.*, 2016).**

Most topical fluoride products rely on patient compliance and render some of these methods less efficacious. The topical fluoride delivery methods such as application of varnish and other remineralizing agents provide adequate control and reduce the need for patient compliance **(Singh *et al.*, 2016).**

Fluoride is the most important remineralizing agent for prevention of dental caries. Different mechanisms have been proposed for the role of fluoride in remineralization but the most important mechanism is the formation of fluorohydroxyapatite crystals which are larger and more resistant to acid dissolution than normal hydroxyapatite crystals **(Amaechi *et al*., 2015)**.

Fluoride has some adverse effects especially when consumed in excessive amounts. Fluorosis is one major concern regarding the use of fluoride**.** Fluoride can pass through the cell membrane and affect the function of a wide range of cell types. Fluoride overdose can irritate

the digestive system and lower the IQ of children **(Bilgin Gocmen *et al.*, 2016)**.

However, the effectiveness of fluoride to promote remineralization is limited by the availability of calcium and phosphate in saliva. Therefore, further preventive measures are required besides fluoride therapy to control the caries process in high caries-risk individuals **(Featherstone and Doméjean , 2012)**.

Theobromine, a safer alternative to fluoride, forms enlarged hydroxyapatite crystallites in the calcium and phosphate rich medium. This reinforces enamel and makes it less vulnerable to acid attacks. Further, theobromine proved to have a great effect on streptococcus mutans **(Premnath *et al.*, 2019).**

Theobromine is an alkaloid belonging to the methylxanthines. It is mainly found in cocoa and chocolate. Theobromine levels are highest in dark chocolate and it is also present in tea and cola nuts. In chocolate, theobromine exists in doses that are safe for humans to consume in large quantities **(Duraisamy *et al.*, 2018).**

Theobromine contained in the cocoa extract will increase the hardness of tooth enamel through interstitial reaction as a substitution for the loss of hydroxyl apatite crystals. Theobromine crystals are smaller than hydroxyapatite crystals will make it easier to get into the tunnel and replace ions in the composition of the apatite. The ion replacement will alter the physical properties of apatite **(Sulistianingsih *et al*., 2017).**

Sadeghpour *et al*, 2007, has conducted a study on animals which reveals that theobromine was more effective in preventing caries compared to fluoride. Application of theobromine to the enamel surface will increase the size of hydroxyapatite crystals that will improve enamel resistance to acids so as to prevent demineralization. The tooth enamel surface hardness can be affected by the exchange of minerals on the surface of the enamel.

Lakshmi *et al*., 2019, have stated that the anticariogenic effect of theobromine is determined by examining its ability to cause remineralization of enamel lesion and concluded that theobromine-forming medium can enhance the remineralization potential of the tooth. The amount of theobromine in a 1-ounce (28.3 grams) dark chocolate bar has a better effect on tooth hardness than a 1.1% prescription sodium fluoride treatment. However, fluoride has so many benefits such as increasing enamel strength and resilience; there is drawback of highly toxic if ingested or absorbed. There has never been a viable alternative to fluoride up until now**.**

It has been demonstrated that the application of theobromine can result in remineralization and prevention of surface caries in enamel

(Durhan *et al.*, 2021). Therefore, the aim of the current study is to evaluate the effect of theobromine varnish on remineralization, microhardness and color change of enamel versus that of fluoride varnish on extracted primary molars.

Research Hypothesis

Theobromine varnish is as effective as fluoride vanish as regards the remineralization, microhardness and color change of enamel of primary molars.

II. MATERIALS AND METHODS

Approvals and Committees

The study was carried on after being reviewed and approved by the Evidence Based Committee, Faculty of Dentistry, Cairo University, the board pf Pediatric Dentistry Department, Faculty of Dentistry, Cairo University and the Research Ethics Committees (REC) of the Faculty of Dentistry, Cairo University, Egypt (No: 2-4-21).

Sample Size calculation

Up to our best knowledge, the theobromine varnish has never been used before on extracted artificially induced carious primary molars, therefore a pilot study was recommended, According to the previous researchers **(Isaac, S. & Michael, W.B., 1995),** who stated that "In cases of exploratory research and pilot studies. Sample sizes of 10 to 30 are sufficient in these cases". Therefore, ten extracted artificially induced carious primary molars were selected and sectioned mesio-distally into two halves. The intervention group (A) (10 halves) were treated with theobromine varnish while the control group (B) (10 halves) received fluoride varnish.

Study Settings

The study setting was conducted in Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University, Egypt.

SEM/EDXA and microhardness measurements analysis were conducted at the National Research Centre (NRC), Egypt. Color change measurement was conducted using Vita Easy Shade device at Faculty of Dentistry, Cairo University, Egypt.

Extracted teeth fulfilling the following criteria were included:

- Primary molars
- Sound enamel surface

Sequence generation was done on the internet by random.org website which gives true random numbers. The samples were given numbers representing samples' allocation into 2 groups (Test group (A) and control group (B)).

The study was performed using samples in the form of 10 primary human molars extracted at the clinic of Pediatric Dentistry Department, Faculty of Dentistry, MSA University. Each tooth was sectioned mesiodistally using diamond disk with copious water coolant to avoid heat generation. Twenty halves of the sectioned teeth were divided into two equal groups. A sample of 10 halves allocated to test group (A) receiving theobromine varnish (test group, n=10) and ontrol group B receiving fluoride varnish (control group, n=10).

Teeth were then mounted on acrylic blocks to be ready. Teeth containers were then randomly allocated either to the control or intervention group.

Test group (A) primary molars were coated with theobromine varnish [1]while control group (B) were coated with fluoride varnish.[2]

Initiation of caries- like lesions

[1] Powder of theobromine (Sigma international company, USA), that are transformed into theobromine varnish by the help of a nanotechnology company (NanoGate Company, Egypt).

[2] 3M™ Clinpro™ White Varnish Desensitizing Varnish with TCP (Tri-Calcium Phosphate) is a proprietary formula that contains fluoride, calcium and phosphate-components naturally found in saliva, 3M company, USA.

The teeth were subjected to demineralization protocol to initiate caries-like lesion; samples were immersed for four days in demineralizing solution (2.2mM Calcium Chloride, 50 mM Acetic Acid and 2.2 mM Sodium Di-hydrogen Phosphate. The pH of the solution was adjusted around 4.5 by the addition of potassium hydroxide).

After initiation of the caries-like lesions, the samples were subjected to pH cycle for five days.

According to Argenta et al., 2003, the pH cycle used was:

1-Application of the treatment material (theobromine or fluoride) for a period = 3 minutes

2-Immersion in demineralization solution for 3 hours.

3-Application of the treatment material (theobromine or fluoride) = 3 minutes.

4-Immersion in 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.

Samples Preparation for SEM/EDXA Analysis:

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

High resolution scanning electron microscopy and qualitative analysis of surface mineral content of enamel samples using EDXA[1], were performed to chemically analyze and measure calcium (Ca) and phosphorous (P) atomic % of each enamel sample under magnifications 1500x ,3000x and 6000x to cover wide fixed area.

The enamel surface topography and surface mineral contents of the samples were assessed using SEM attached with EDXA Unit with magnification 1500x, 3000x, 6000x at baseline, after demineralization and after remineralization. Three scans were made at each location and the three readings were averaged.

The data of calcium (Ca) and phosphorous (P) percentage was tabulated and the Ca/P ratio was calculated as an indication of the mineral changes during demineralization and remineralization.

Sample Preparation for Microhardness tester:

The microhardness of all the samples were tested using universal testing machine (Vicker's microhardness tester)[2]. The samples were tested at 100 gf, 30s dwell time, at magnification 40x. Baseline, demineralization and remineralization readings of the samples were recorded.

Sample preparation for Color change measurement:

The effect of theobromine varnish and fluoride varnish on the color change of enamel was measured using a color assessment method which is a digital spectrophotometer (Vita Easy Shade V). Samples were measured for color change at baseline, after demineralization and after remineralization.

The Easy Shade Spectrophotometer calculates CIE L*a*b* values where:

L* is a measure of the lightness- darkness of the sample, a* represents the redness (positive value) or the greenness (negative value), b* is a measure of the yellowness (positive value) or the blueness (negative value).

Then, the color changes ΔE were calculated based on the CIE L*a*b* color system.

Statistical Analysis:

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution, and using Shapiro-Wilk test.

Data showed parametric distribution and were analyzed using independent t-test for intergroup comparisons and repeated measures

[1] SEM Quanta FEG 250 with field emission gun, FEI Company − Netherlands).
[2] (NEXUS 4000TM, model no. 4503, INNOVATEST Europe BV, Maastricht, Netherlands)

ANOVA followed by Bonferroni post hoc test for intragroup comparisons.

The significance level was set at $p<0.05$. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Window **(R Core Team, 2022).**

III. RESULTS

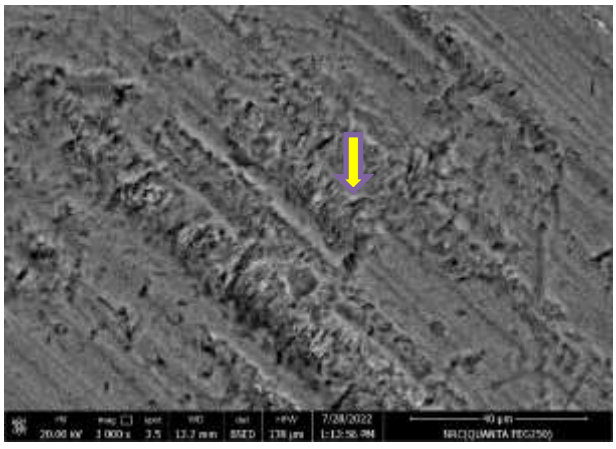
Scanning Electron Microscope (SEM) Examination:

The Scanning Electron Microscope pictures gave indicators of the quality of the tested varnishes, during different investigation periods. Pictures were taken with magnification 1500X, 3000X and 6000X.

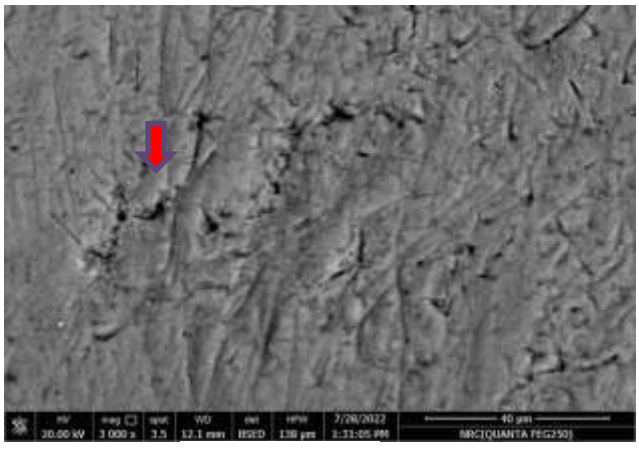
At baseline, the sound enamel showed the same characteristics and features which are smooth surface with relatively variable appearance such as prismatic enamel, perikymata, cracks, pits and focal holes, figure (1).

After demineralization, in both groups the demineralized pattern was the same. The demineralization significantly altered the prismatic structure of sound enamel. All samples showed alteration in the prismatic structure of sound enamel with varying degrees of dissolution areas. Demineralization is represented by pores and is characterized by typical honeycomb or fish scales appearance, figure (2).

After remineralization, the demineralized enamel surfaces treated with both varnishes showed partial restoration of the surface structure of the enamel which can be observed by considerable disappearance of dissolution areas with minimizing the porosity size indicating remineralization. In both, test group and the control group, the enamel surface showed areas of uniform and smooth enamel surface. In addition, some prism cores had been completely obliterated and not remarkably visualized, figures (3) and (4).

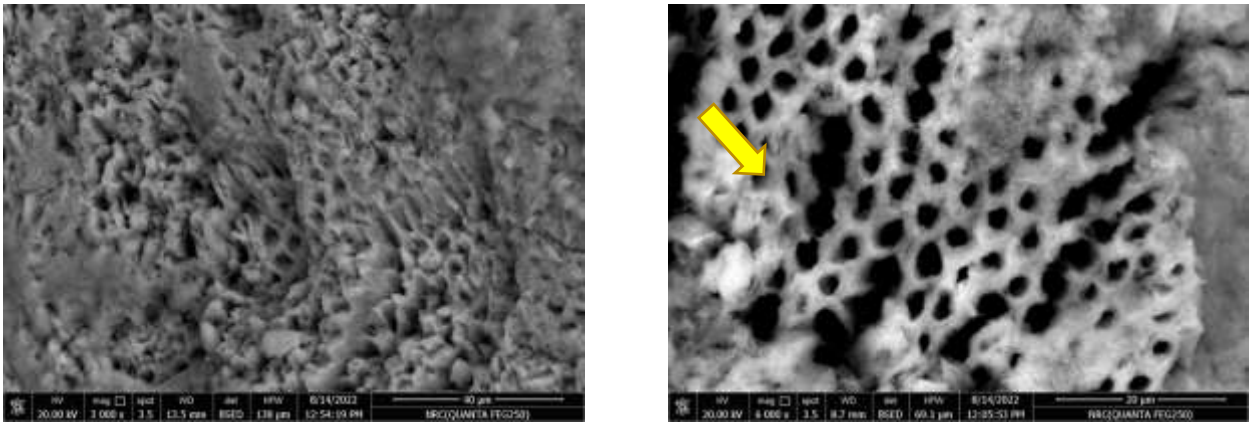


(a)



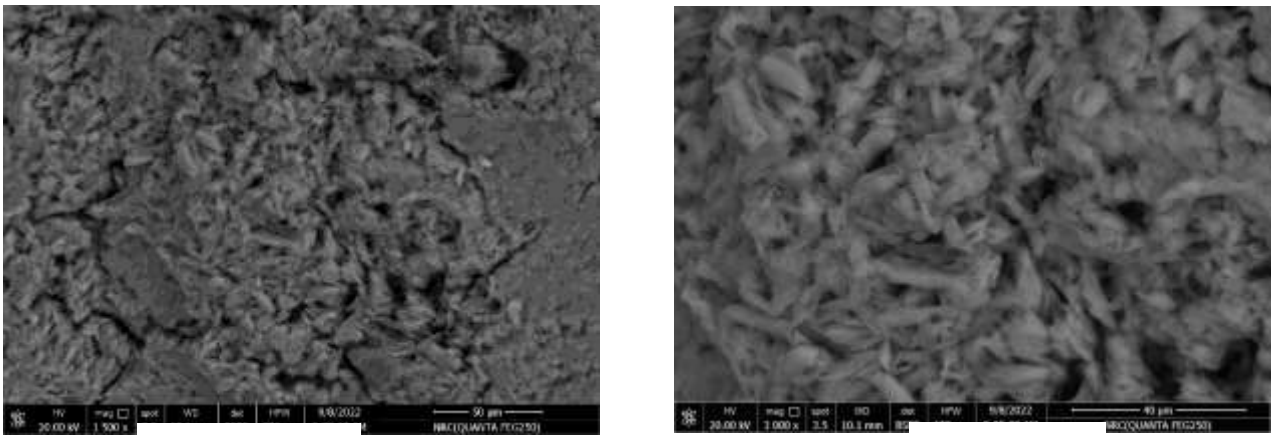
(b)

Figure (1): SEM photomicrograph of enamel specimen at baseline a magnification 3000x showing (a) perikymata (yellow arrow), (b) focal holes (red arrow)

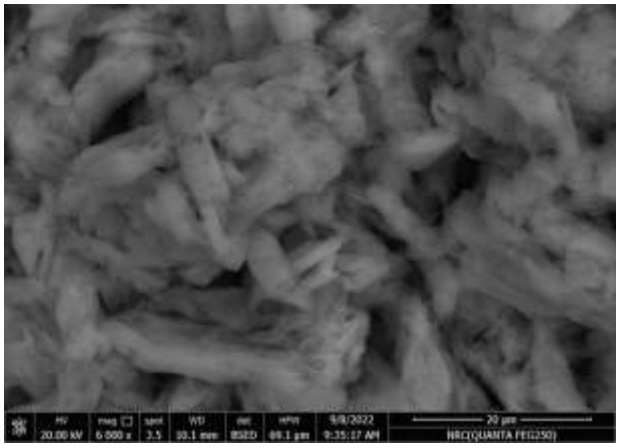


(a) (b)

Figure (2): SEM photomicrograph of demineralized enamel specimen (a) at magnification 3000x showing honeycomb appearance with erosion of prism cores and preserved prism peripheries, (b) at magnification 6000x showing dissolution voids (yellow arrow)

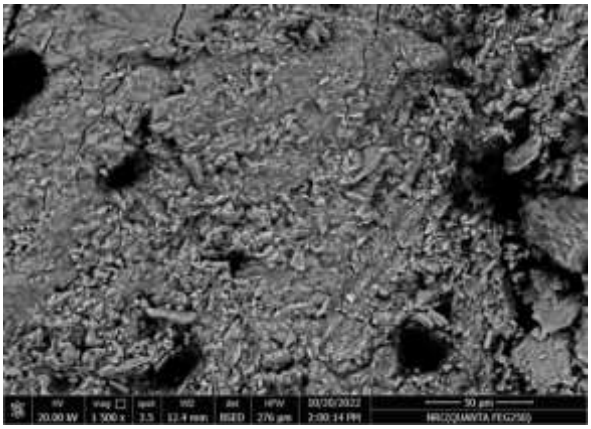


(a) (b)

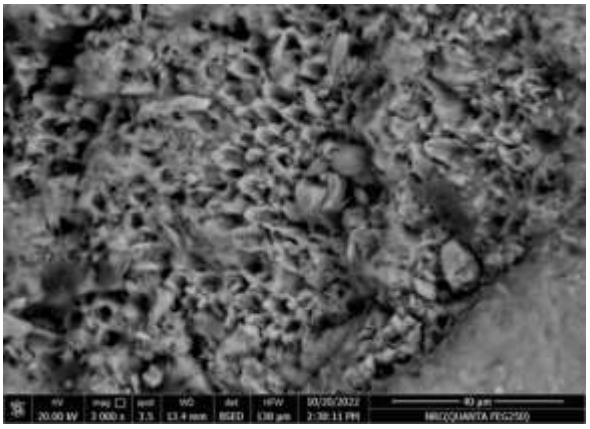


(c)

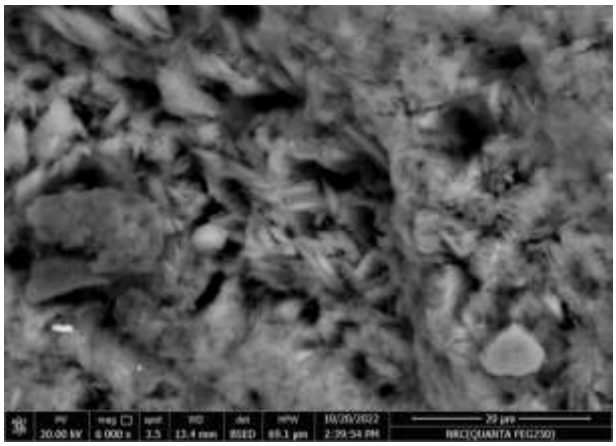
Figure (3): SEM photomicrographs of enamel specimens treated with theobromine varnish (test group) at magnifications (a) 1500x, (b) 3000x, (c) 6000x showing areas of calcified deposits. Amorphous calcific deposits were scattered on the enamel surface along the prismatic borders indicating remineralization.



(a)



(b)



(c)

Figure (4): SEM photomicrographs of enamel surface treated with fluoride varnish (control group) with magnifications (a) 1500x, (b) 3000x and (c) 6000x showing partial restoration of the surface structure of enamel which can be observed by considerable disappearance of dissolution areas with minimizing the porosity.

EDXA analysis

Calcium

Intergroup comparisons

At all intervals (baseline, demineralization and remineralization), there was no significant difference between both groups ($p>0.05$) in the calcium content. At baseline, afterdemineralization and after remineralization, both the test and control groups showed no significant difference in the calcium content, table (1).

Intragroup comparisons

For both groups, there was no significant difference between values measured at different intervals ($p>0.05$). At baseline, after demineralization and after remineralization there was no significant difference between values of calcium content, table (1).

Phosphorus:

Intergroup comparisons

At baseline and after demineralization, both the test and control groups showed no significant difference in the phosphorous content.

After remineralization, the control group had significantly higher value than the test group ($p=0.002$). For other intervals, the difference was not statistically significant ($p>0.05$), table (1).

Intragroup comparisons

For both groups, there was no significant difference between values measured at different intervals ($p>0.05$).

At baseline, after demineralization and after remineralization, there was no significant difference between values of phosphorous content, table (1).

Ca/P

Intergroup comparisons

At all intervals, there was no significant difference between both groups ($p>0.05$), table (1).

Intragroup comparisons

For the test group, there was a significant difference between values measured at different intervals ($p=0.018$) and post hoc pairwise comparisons showed enamel value to be significantly higher than value measured after remineralization ($p<0.001$). For the control group, there was no significant difference between values measured at different intervals ($p=0.064$), table (1).

Table (1): Inter and intragroup comparisons, mean and standard deviation (SD) values of weight percentage (%)

Element	Interval	Weight percentage (%) (mean±SD)		lue
		Test group	Control group	
Calcium	Enamel	23.35±3.23^{A}	23.29±2.60^{A}	**0.975ns**
	Demineralization	20.63±1.17^{A}	20.84±2.64^{A}	**0.878ns**
	Remineralization	22.20±3.37^{A}	22.60±1.70^{A}	**0.819ns**
	p-value	**0.348ns**	**0.291ns**	
Phosphorus	Enamel	13.59±1.51^{A}	13.81±1.06^{A}	**0.797ns**
	Demineralization	12.89±0.66^{A}	13.14±1.08^{A}	**0.678ns**
	Remineralization	12.65±0.39^{A}	13.45±0.01^{A}	**0.002***
	p-value	**0.340ns**	**0.509ns**	
Ca/P	Enamel	1.74±0.03^{A}	1.68±0.07^{A}	**0.157ns**
	Demineralization	1.62±0.03^{AB}	1.58±0.08^{A}	**0.347ns**
	Remineralization	1.65±0.08^{B}	1.67±0.02^{A}	**0.534ns**
	p-value	**0.018***	**0.064ns**	

Means with different superscript letters within the same vertical column are significantly different *; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

Micro-hardness

Intergroup comparisons

After remineralization, the test group had significantly higher value than the control group (p=0.014). For other intervals, the difference

was not statistically significant (p>0.05), table (2).

Intragroup comparisons

For both groups, there was a significant difference between values measured at different intervals (p<0.001). For the test group, post hoc pairwise comparisons showed remineralization value to be significantly higher than other intervals (p<0.001). In addition, they showed enamel value to be significantly higher than demineralization value (p<0.001).

For the control group, the comparisons showed remineralization and enamel values to be significantly higher than demineralization values (p<0.001), table (2).

Table (2): Inter and intragroup comparisons, mean and standard deviation (SD) values of micro-hardness

Interval	Micro-hardness (mean±SD)		p-value
	Test group	Control group	
Enamel	134.14±8.94^{B}	131.24±6.47^{A}	**0.573ns**
Demineralization	93.71±1.70^{C}	95.67±3.25^{B}	**0.267ns**
Remineralization	148.75±4.73^{A}	136.66±7.15^{A}	**0.014***
p-value	**<0.001***	**<0.001***	

Means with different superscript letters within the same vertical column are significantly different *; significant ($p \leq 0.05$) ns; non-significant (p>0.05)

Color change (ΔE)

Inter and intragroup comparisons

At baseline, after demineralization and after remineralization,, there was no significant difference between both groups (p>0.05) , table (3).

Table (3): Inter and intragroup comparisons, mean and standard deviation (SD) values of color change (ΔE)

Interval	Color change (ΔE) (mean±SD)		p-value
	Test group	Control group	
Enamel – remineralization	10.49±2.30	12.14±2.95	**0.180ns**
Demineralization- remineralization	9.75±1.97	11.02±2.86	**0.288ns**

IV. DISCUSSION

Caries prevention is considered one of the most important goals in the dental field especially in pediatric dentistry. The present study focused on the remineralization as an effective tool for reversing incipient caries. Although the enamel remineralization potential and its role in caries arresting are widely discussed in the literature, there is controversy about the categorization of the remineralizing materials as well as the techniques for application of these materials.

Two remineralizing materials were selected; the first was 3M™ Clinpro™ white desensitizing varnish which is considered for long time the most effective method of caries prevention. The second is theobromine varnish, a promising caries preventive material comparable to fluoride but safer than fluoride as theobromine is non-toxic.

To strengthen our conclusions, the performance of theobromine was compared with that of a material that has long been its substitute in the market: sodium fluoride varnish. The use of theobromine as a remineralization agent was based on research by **Nakamoto *et al*., 2016**, that showed theobromine and fluoride are two substances that can elevate apatite crystal size, which is related to enamel surface hardness. **Nakamoto *et al*., 2016**, stated that theobromine is safer because of its low toxicity level when compared to fluoride.

The initiation of enamel caries like lesions in our work was performed by chemical in-vitro models due to their simplicity, low cost, and experimental stability

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. In the present work, after initiation of caries like lesions, the PH cycling models were used to mimic the in vivo periodic alternation of PH.

In the current study, the concentration of theobromine used is 200 mg/l based on the results concluded by **Kargul *et al*., 2012,** where

the surface microhardness values showed that 200 mg/l theobromine protected enamel specimens more than 100 mg/l theobromine. Also, the enamel specimens treated with 100 mg/l theobromine showed less smooth surface with lines and pits. The research demonstrated 200 mg/l theobromine to be more effective at increasing enamel surface hardness.

Up to our best knowledge, this is the first study to use theobromine powder, convert it into varnish by the aid of a nanotechnology company (NanoGate) & compare its remineralization effect, micohardness effect and color change on enamel versus that of the gold standard sodium fluoride varnish.

SEM was selected for assessment since it is one of the most sensitive in vitro methods to evaluate the demineralization and remineralization processes. It gives detailed high resolution images of the specimens. Furthermore, EDXA analysis was performed to provide elemental recognition and quantitative information about the composition**.**

The results presented porous enamel surface after demineralization. This coincides with **Karlinsey *et al.*, 2012**, who reported that the acid application dissolves calcium and phosphate ions which cause gapping between crystals which, in turn, lead to enamel porosity. 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**.**

In the present study, EDXA revealed more improved calcium in the theobromine varnish group than in the fluoride group, but these results were statistically non-significant ($p > 0.05$). Similar results were reported by **Amaechi *et al*., 2013** and **Nakamoto *et al*., 2016**.

However, the results are in disagreement with the results of **Lippert *et al*., 2017**, who compared the remineralization potential of fluoride, theobromine and their combinations on demineralized carious lesions and concluded that theobromine did not provide remineralization under the selected conditions.

SEM images showed that theobromine also gave smooth surface of the remineralized enamel which is close to the normal surface appearance before demineralization which is similar to the results reported by **Taneja *et al*., 2019**.

Microhardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth. This method is easy, quick, and requires only a small area of specimen surface for testing. The commonly used microhardness tests

for evaluating enamel remineralization are Vickers' microhardness test and Knoop microhardness test.

Sadeghpour *et al.*, 2007, stated that theobromine causes calcium and phosphate to merge into a crystal unit that is four times bigger than hydroxyapatite. Our results revealed that although theobromine varnish caused increase in the Ca/P ratio but did not 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.

After remineralization, theobromine varnish group had significantly higher value of microhardness than the fluoride varnish group which is similar to the results reported by **Irawan *et al.*, 2017, Sulistianingsih *et al.*, 2017** and **Suryana *et al.*, 2018**.

The color changes of enamel before, after demineralization and after remineralization were measured using the digital spectrophotometer, Vita EasyShade. According to the conclusions of **Knezović *et al.*, 2015** studie**s,** accuracy of the shade-matching device was very high. VITA Easyshade® Advance 4.0 dental shade-matching device enabled reliable and accurate measurement. It can be a valuable tool for the determination of tooth colors.

Based on the results of this study, there was no significant difference between theobromine varnish and fluoride varnish in changing the color of primary teeth enamel ($p > 0.05$). These results showed theobromine and fluoride had similar effects in preventing discoloration which is similar to the results published by **Wulandari *et al.*, 2018.**

V. CONCLUSIONS

From the results of the present study, the following can be concluded:

1. Theobromine has comparable remineralization potential as fluoride.
2. Theobromine can improve the remineralization potential of medium rich in calcium and phosphate.
3. Theobromine applied to enamel produces smooth surface through remineralization and does not affect color change similar to fluoride varnish.

VI. LIMITATIONS

The limitations of this study were:

1. The present study did not incorporate a cariogenic biofilm. There is emerging evidence which shows that theobromine can impair the growth of a cariogenic biofilm. Future studies need to explore this further by studying enamel and dentin demineralizing and remineralization using microbial models.
2. The study is in-vitro which is carried out on extracted primary molars. In-vivo studies are recommended.

VII. RECOMMENDATIONS

From the results of the present study, the following can be recommended:

1. Theobromine is a nontoxic, natural, and an effective remineralizing agent that could be suitable alternative to fluoride.
2. Further studies are recommended to support the fact that theobromine can be a valuable substitute to fluoride in commercial dentifrices.

REFERENCES

Amaechi, B. T. (2015). Remineralization therapies for initial caries lesions. Current Oral Health Reports, 2(2), 95-101.

Amaechi, B. T., Porteous, N., Ramalingam, K., Mensinkai, P. K., Vasquez, R. C., Sadeghpour, A., & Nakamoto, T. (2013). Remineralization of artificial enamel lesions by theobromine. Caries Research, 47(5), 399-405.

Argenta, R. M. O., Tabchoury, C. P. M., & Cury, J. A. (2003). A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. Pesquisa Odontológica Brasileira, 17, 241-246.

Durhan, M. A., Bilsel, S. O., Gokkaya, B., Yildiz, P. K., & Kargul, B. (2021). Caries Preventive Effects of Theobromine Containing Toothpaste on Early Childhood Caries: Preliminary Results. Acta Stomatologica Croatica, 55(1).

Featherstone, J. D. (2008). Dental caries: a dynamic disease process. Australian dental journal, 53(3), 286-291.

Featherstone, J. D., & Doméjean, S. (2012). The role of remineralizing and anticaries agents in caries management. Advances in dental research, 24(2), 28-31.

Gocmen, G. B., Yanikoglu, F., Tagtekin, D., Stookey, G. K., Schemehorn, B. R., & Hayran, O. (2016). Effectiveness of some herbals on initial enamel caries lesion. Asian Pacific Journal of Tropical Biomedicine, 6(10), 846-850.

Hammad, S. M., El-Wassefy, N. A., & Alsayed, M. A. (2020). Evaluation of color changes of white spot lesions treated with three different treatment approaches: an in-vitro study. Dental press journal of orthodontics, 25, 26-27.

Irawan, M. I. P., Noerdin, A., & Eriwati, Y. K. (2017, August). The effect of time in the exposure of theobromine gel to enamel and surface hardness after demineralization with 1% citric acid. In Journal of Physics: Conference Series, 884(1), 012005.

Kargul, B., Özcan, M., Peker, S., Nakamoto, T., Simmons, W. B., & Falster, A. U. (2012). Evaluation of human enamel surfaces treated

with theobromine: a pilot study. Oral Health and Preventive Dentistry, 10(3), 275.

Karlinsey, R. L., Mackey, A. C., Walker, E. R., & Frederick, K. E. (2010). Preparation, characterization and in vitro efficacy of an acid-modified β-TCP material for dental hard-tissue remineralization. Acta Biomaterialia, 6(3), 969-978.

Knezović, D., Zlatarić, D., Illeš, I. Ž., & Alajbeg, M. (2015). In vivo and in vitro evaluations of repeatability and accuracy of VITA Easyshade® Advance 4.0 dental shade-matching device. Acta Stomatologica Croatica, 49(2), 112.

Lakshmi, A., Vishnurekha, C., & Baghkomeh, P. N. (2019). Effect of theobromine in antimicrobial activity: An in vitro study. Dental research journal, 16(2), 76.

Lippert, F. (2017). The effects of fluoride, strontium, theobromine and their combinations on caries lesion rehardening and fluoridation. Archives of Oral Biology, 80, 217-221.

Nakamoto, T., Falster, A. U., & Simmons Jr, W. B. (2016). Theobromine: a safe and effective alternative for fluoride in dentifrices. Journal of caffeine research, 6(1), 1-9.

Paula, A. B. P., Fernandes, A. R., Coelho, A. S., Marto, C. M., Ferreira, M. M., Caramelo, F., ... & Carrilho, E. (2017). Therapies for white spot lesions—a systematic review. Journal of Evidence Based Dental Practice, 17(1), 23-3

Singh, S., Singh, S. P., Goyal, A., Utreja, A. K., & Jena, A. K. (2016). Effects of various remineralizing agents on the outcome of post-

Suryana, M., Irawan, B., & Soufyan, A. (2018, August). The effects of toothpastes containing theobromine and hydroxyapatite on enamel orthodontic white spot lesions (WSLs): a clinical trial. Progress in orthodontics, 17(1), 1-8.

Premnath, P., John, J., Manchery, N., Subbiah, G. K., Nagappan, N., & Subramani, P. (2019). Effectiveness of theobromine on enamel remineralization: a comparative in-vitro study. Cureus, 11(9).

Roopa, K. B., Pathak, S., Poornima, P., & Neena, I. E. (2015). White spot lesions: A literature review. Journal of Paediatric Dentistry, 3(1), 1-7

Sadeghpour, A. (2007). 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, 68(07).

Veiga, N. J., Aires, D., Douglas, F., Pereira, M., Vaz, A., Rama, L., ... & Bexiga, F. (2016). Dental caries: A review. Journal of Dental and Oral Health, 2(5), 1-3.

Wulandari, N. Y., Irawan, B., & Herda, E. (2018, August). Effects of theobromine toothpaste on prevention of enamel discoloration from coffee. In Journal of Physics: Conference Series, 1073(3), 032009.

microhardness after immersion in carbonated drink. In Journal of Physics: Conference Series, 1073(3), 032010.