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Original Article

The prophylactic effect of Theobromine on enamel de-mineralization induced by pop candies on extracted deciduous and permanent human teeth: In vitro study.

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Abstract

Aim: The aim of this study was to evaluate the prophylactic effect of Theobromine on enamel de-mineralization induced by pop candies on extracted deciduous and permanent human teeth.

Subjects and methods: Twenty permanent premolars and twenty primary molars were selected. The apical foramen of each tooth was blocked with pink wax to prevent fluid entrance. The incisal and middle thirds were coated by nail polish. Three grams of popping candies were purchased, crushed, and then dissolved in 2 mL of artificial saliva. Teeth were randomly divided into four groups:

1. Group A (-ve control group): no application of pop candies nor Theobromine.
2. Group B (+ve control group): application of pop candies only.
3. Group C (experimental group): application of Theobromine before and after emersion in pop-candies solution.
4. Group D (experimental group): application of Theobromine after emersion in pop candies solution.

For the two experimental groups C and D; one drop of Theobromine will be applied on tooth surface for 5 minutes in 5 days, then immersed in the artificial saliva until the next stage of the test. The two groups were exposed to pop candies solution of artificial saliva once daily for 5 min in 5 days. One drop of Theobromine was applied on the enamel surface for 2 minutes after each exposure for group D.

Results: After demineralization Group B, the surface of exposed enamel showed rough texture, bright white in color. The second group, Group C showed the maximal changes as the surface became smoother and the enamel color almost like normal enamel. The third group, Group D showed an average change in both surface texture and color. Group C showed a higher mean value than Group D as theobromine was used before and after de-mineralization.

Conclusion: Theobromine is not only a good re-mineralizing agent, but also it can interfere and inhibit the demineralization process and decrease mineral loss.

Keywords: Enamel, Re-mineralization, Theobromine, Pop candies, De-mineralization.

Introduction

Enamel is the outermost covering of vertebrate teeth and the hardest tissue in the vertebrate body. It has a unique morphological structure and distinctive mechanical properties, making it different from other mineralized tissues such as the bone and dentin in the human body. It is composed of 95-97% highly organized hexagonal carbonated hydroxyapatite (HA) crystals $Ca_{10}(PO_4)_6(OH)_2$ by weight. These crystals are roughly parallel to form highly organized architectural units known as enamel rods. Enamel thickness and translucency are responsible for its esthetical appearance. The unique shapes and organizations of enamel crystals determine the excellent mechanical properties of tooth enamel with increased hardness and resistance to fracture and acid erosion (Cao *et al* ., 2014; Desouky *et al*., 2022).

Enamel is secreted only once prior to tooth eruption, and the capacity to form new enamel in each individual tooth organ is lost forever, once the tooth is fully erupted. So, re-mineralization is mandatory process for keeping that precious hard tissue (Schroeder and Listgarten, 1971).

Dental erosion is a common disease with increasing prevalence in recent years. (Salas *et al*., 2015). It occurs because of a chemo-mechanical process involving intrinsic or extrinsic acids of non-bacterial origin and mechanical forces, such as attrition and abrasion. (Carvalho *et al*., 2015; Shellis *et al*., 2014).

Early erosive damage to teeth may cause severe tooth surface loss, tooth sensitivity, over closure, and poor esthetic; The advanced stages of this condition may impair esthetics and function, affecting the patient's quality of life; (Papagianni *et al*., 2013) Therefore, early diagnosis of the disease is an appropriate preventive measure and is very important (Monika *et al*., 2009).

The preventive management of erosion should start as early as possible and focus on intercepting causal factors such as dietary habits, gastroesophageal reflux, general medical conditions, oral hygiene habits, and functional problems (Carvalho *et al*., 2015).

The final erosive potential of food and drinks depends on the contrast between: chemical properties (pH, total acidity, calcium and the amount of phosphate, and adhesion), biological factors (salivary flow rate, buffering capacity and composition, pellicle formation, tooth composition, and soft-tissue anatomy), and behavioral factors (eating and drinking habits, especially continuous and prolonged ones) (Monika, 2009).

Popping candies are produced from a mixture of sucrose, lactose, and corn starch dissolved in water, and then, the solution is melted to the point where it includes 2%–3% water, and then, carbon dioxide (CO2) is exposed to molten sugar at high pressure (625–675 pressure), followed by the mixture being cooled. When the candies are kept inside the mouth, they melt and gas escapes which creates short feelings of entertainment. (Kleiner *et al*., 1981). Pop candies were used due to the high sugar content which induce enamel demineralization.

One key factor in the salivary mechanism for erosion protection is the formation of the acquired enamel pellicle (AEP) (Hannig *et al*., 2012) which protects the enamel surface from direct contact with acids, reducing and retarding enamel demineralization. (Amaechi, 1999). However, the AEP cannot protect against severe erosive challenges; (Hara *et al*., 2006) As about 25% of the acquired pellicle dry weight is lipid, lipophilic components (Hannig *et al*., 2003) can modulate the composition and ultrastructure of the pellicle; Thus, it has been speculated that lipid-enriched

pellicles are more resistant to acids and might hinder dental erosion. (Kensche *et al*., 2013).

Re-mineralization of enamel is a well-accepted preventive concept for maintaining enamel integrity. Different systems of re-mineralization have been investigated to achieve the goal of building up enamel including; Fluoride varnishes, amorphous calcium phosphate, re-mineralizing water-based cream containing hydroxyapatite, fluoride and xylitol, micro-structure; calcium phosphate nanoparticles, amyloid like peptides, enamel matrix derivatives and other organic materials (Husain *et al* ., 2017 ; Buzalaf , 2018 ; Shihabi *et al*., 2021).

Theobromine (3,7-dimethylxanthine) is a primary alkaloid derived from cacao plant. It is a water-soluble, crystalline, bitter powder found in chocolates along with tea and other foods. Although consumption of chocolate which is processed from cacao seeds has been associated with high incidence of dental caries (Irawan *et al*., 2017). Recently, studies have been made for using theobromine as an effective re-mineralizing agent and can be used as an alternative to fluorides (Irawan *et al., 2017,* Amaechi *et al., 2013).* Theobromine, in the presence of calcium and phosphate, forms hydroxyapatite crystallites of an increased size that strengthen the enamel, making it less susceptible to acid attack, which eventually leads to cavitation (Irawan *et al*., 2017*).*

Thus, we aimed to evaluate the effect of theobromine on enamel demineralized by pop candies on deciduous and permanent teeth.

Subjects and Methods

Experimental design:

This study was approved by ethical committee of faculty of Dental Medicine, Cairo University by number 4 6 21. SEM with EDXA was done for both permanent premolar samples and primary molar samples before (baseline), (prophylactic), after demineralization, and after treatment with theobromine.

Preparation of demineralizing solutions:

Johnybee popping candies, UK were supplied online from Amazon. It is produced from a mixture of sucrose, lactose and corn starch. Each candy was crushed with a pestle, and demineralizing solution consisted of 3 grams of each popping candy dissolved in 2 mL of artificial saliva at 37 degrees Celsius and a pH of 5.5. Three grams of Johnybee popping candies contain the following nutritional values: fat is 0.0 gram (thereof saturated fatty acids 0.0 gram), carbohydrates is 2.97 grams (thereof sugar 2.72 gram), protein is 0.0 gram and salt is 0.0 gram

Theobromine:

It was purchased from Penta Manufacturing Company, New Jersey, United states. It if supplied in form of white water-soluble crystalline powder found mainly in cocoa, chocolate and Camellia Sinensis green tea leaves which is a major constituent of cocoa beans and can also be found in dark chocolate. Theobromine is only soluble in water (330 mg/L).

Artificial saliva:

It is supplied as spray called Saseem Mundspray, Germany. It is composed of sodium chloride 0.4 g/lit, potassium chloride 0.4 g/lit, calcium chloride 0.8 g/lit, sodium di hydrogen phosphate 0.78 g/lit, sodium sulfide 0.005 g/lit and Urea 1 g/lit.

Teeth selection:

Twenty permanent premolars were extracted from patients with a range from 15 to 20 years old from orthodontic and surgery departments at faculty of dentistry Misr University for science and technology. Twenty deciduous molars were also collected from the pedodontics department faculty of dentistry Misr University for science and technology. Collected teeth were follow the inclusion

criteria; (Deciduous and young permanent teeth free from caries, no enamel cracks and no developmental defects).

Teeth preparation:

All teeth were cleaned ultrasonically. The occlusal and middle thirds of the crowns were coated by nail polish.

Grouping:

All the extracted permanent and deciduous teeth were randomly divided into 4 groups (A, B, C and D); each group having 5 permanent and 5 deciduous teeth. Group A is a control group, in Group B; the teeth were demineralized using pop candies solution, Group C; the teeth were treated by theobromine before and after emersion in the demineralizing solution, and finally Group D where the teeth were treated by theobromine after demineralization process.

Demineralization:

The three groups B, C and D were exposed to the solution containing 3 g of popping candies dissolved in 2 mL of artificial saliva in 37 degrees Celsius, once daily 5 min for 5 days. Pop candies were used due to the high sugar content which induce enamel demineralization.

Treatment with theobromine:

Even for Group C treatment with theobromine before and after demineralization or Group D treatment with theobromine after demineralization were according to the same protocol. 2 mL of theobromine were added to the surface of the teeth and left for 5 minutes. This procedure was repeated for 5 days.

Scanning electron microscope with EDXA:

The conventional scanning of electron microscope was done to evaluate the surface topography of teeth showing the rod ends that approved the demineralization process, EDX to evaluate the Ca/P ratio.

Statistical analysis:

The data were tabulated, and comparison was done in order to evaluate the effect of using theobromine as prophylactic and re-mineralizing agent. Data were fed to the computer and analyzed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. One- way ANOVA was done to analyze the results.

Results

SEM:

Permanent teeth:

Group A showed smooth surface with simple scratches on the surface and white mineral precipitations. **Group B** the disappearance of prism-less area, also the prisms morphology and confirmed loss of crystals. Loss of crystals was not only in core of prism causing micropores but also in peripheries which made it very difficult to recognize the keyhole or fish scale shape of the normal prism causing widening in the spaces between prisms. In addition to the surface irregularities, porosities, and roughness. **Group C** showed the remineralization as, minerals were not precipitated only in core causing disappearance of micropores in premolars or perspiration in the peripheries regaining the natural appearance of prism shape and the crystal pattern of deposition, but also addition of mineral layer to the surface of the enamel increasing its thickness in an attempt to cover the prism shape to regain the normal surface of enamel with its primeless area. **Group D** remineralization process succeeded in regaining the prism shape as keyhole without loss in the core crystals and also the peripheral crystals by precipitation of minerals forming the prism sheath, leaving no micropores. This sample showed an average remineralization

process. The regular growth of bundles of well-organized crystals was obvious in this group leading to the formation of a continuous compact enamel-like layer as shown in figure 1.

Deciduous teeth:

Group A showed smooth surface with simple scratches and localized pitting effect. **Group B** showed exposed prism like structures, loss of minerals at the core and the peripheries, honey comp appearance, surface irregularities and large diameter micropores. **Group C** the maximal remineralization was found in this group as the micropores were completely closed and the surface appeared to be semi smooth again, the moieties appearance disappeared completely, and layer of minerals covered the prisms' ends. **Group D** showed average remineralization as some craters were found, some irregular spots, even the micropores were filled by minerals precipitations, but the difference between prismatic and interprismatic area is still obvious as shown in figure 2.

EDXA:

Permanent teeth results:

EDX examination of the working groups yields the following descending order of Ca/P ratios. The superiority was for Group C (experimental group) where the ratio was

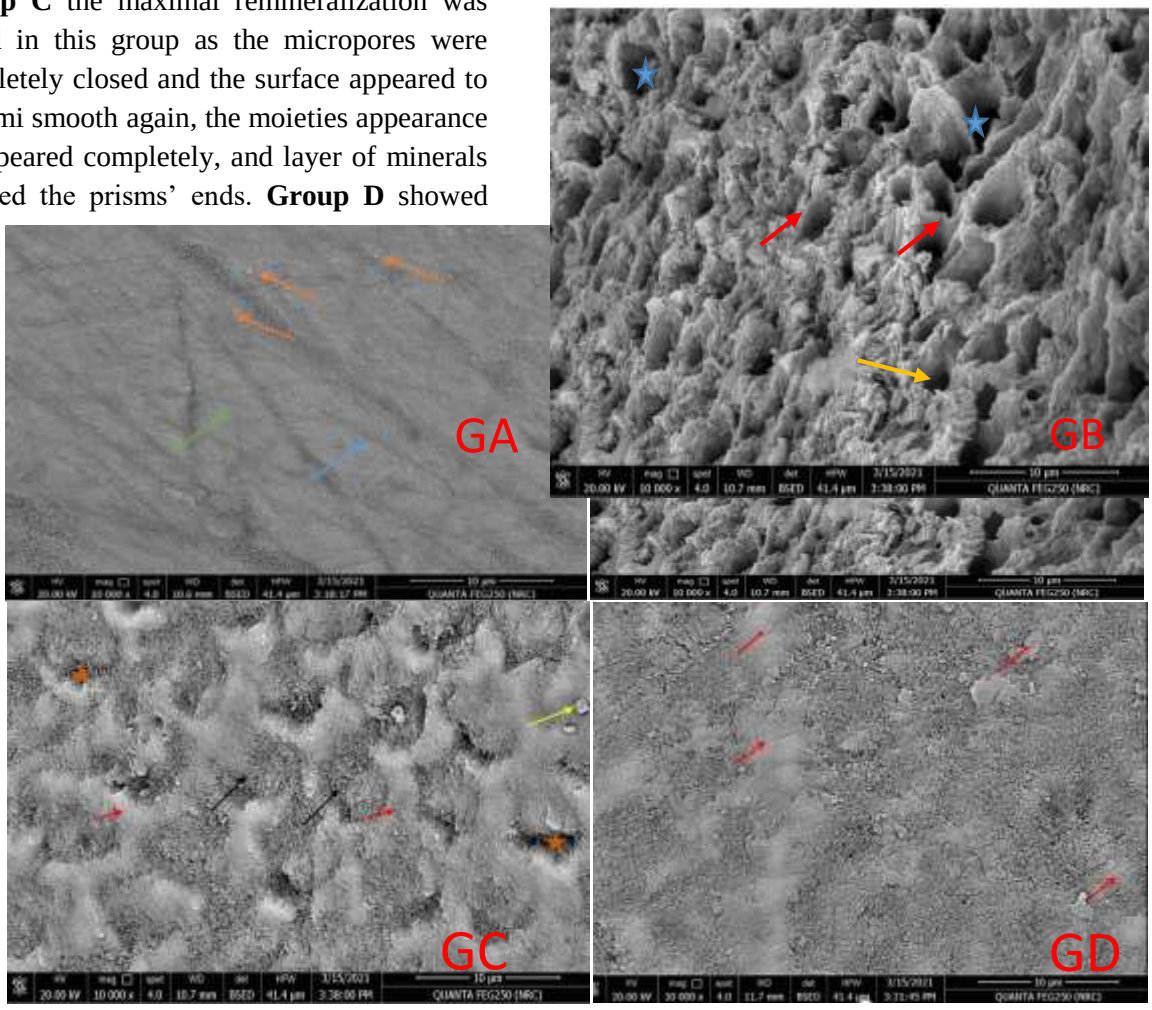


*Figure 1: S*howing scanning of permanent teeth of all groups, GA(-ve control): showing smooth surface with no rod and normal morphology (blue arrow). Several white precipitations of minerals (orange arrows). Simple scratches were seen (green arrow). GB(demineralized): showing more extensive destruction to the crystalline structure of rod end than interprismatic area (yellow arrows). Deep extent of destruction leaving parts of interprismatic enamel (red arrows). It was very difficult to recognize the keyhole shape of the normal prism due to widening in the spaces between prisms(asterisks). In addition to the surface irregularities, porosities (yellow arrows). GC(prophylactic): showing that rod ends appearance was highly significant (black arrows). Obviously, the difference in depth between the higher interprismatic substance and depressed prismatic area was noticed (red arrows). Several white precipitations of inorganic material deposited at the surface (yellow arrows). Countable irregular spots were seen (asterisks). GD (treated): showing that the destructed areas had been fixed and filled by inorganic components (red arrows). But by this magnification the shadows of fish scale appearance or interprismatic areas were difficult to be recognized.

succeeded remineralization process. Group (pop candies group) showed great loss of minerals due to use of pop candies as demineralizing agent. It was reported that mean Ca/P ratio dropped from **2.20 to 1.95** Where the f- value = 46.69, the P- value is ≤ 0.00. So, this result is highly significant as shown in figure 3.

Deciduous teeth results:

Group B (pop candies group) showed great loss of minerals due to use of pop candies as demineralizing agent. It was reported that the mean Ca/P ratio dropped from **1.51 to 1.09**. While Group C showed the

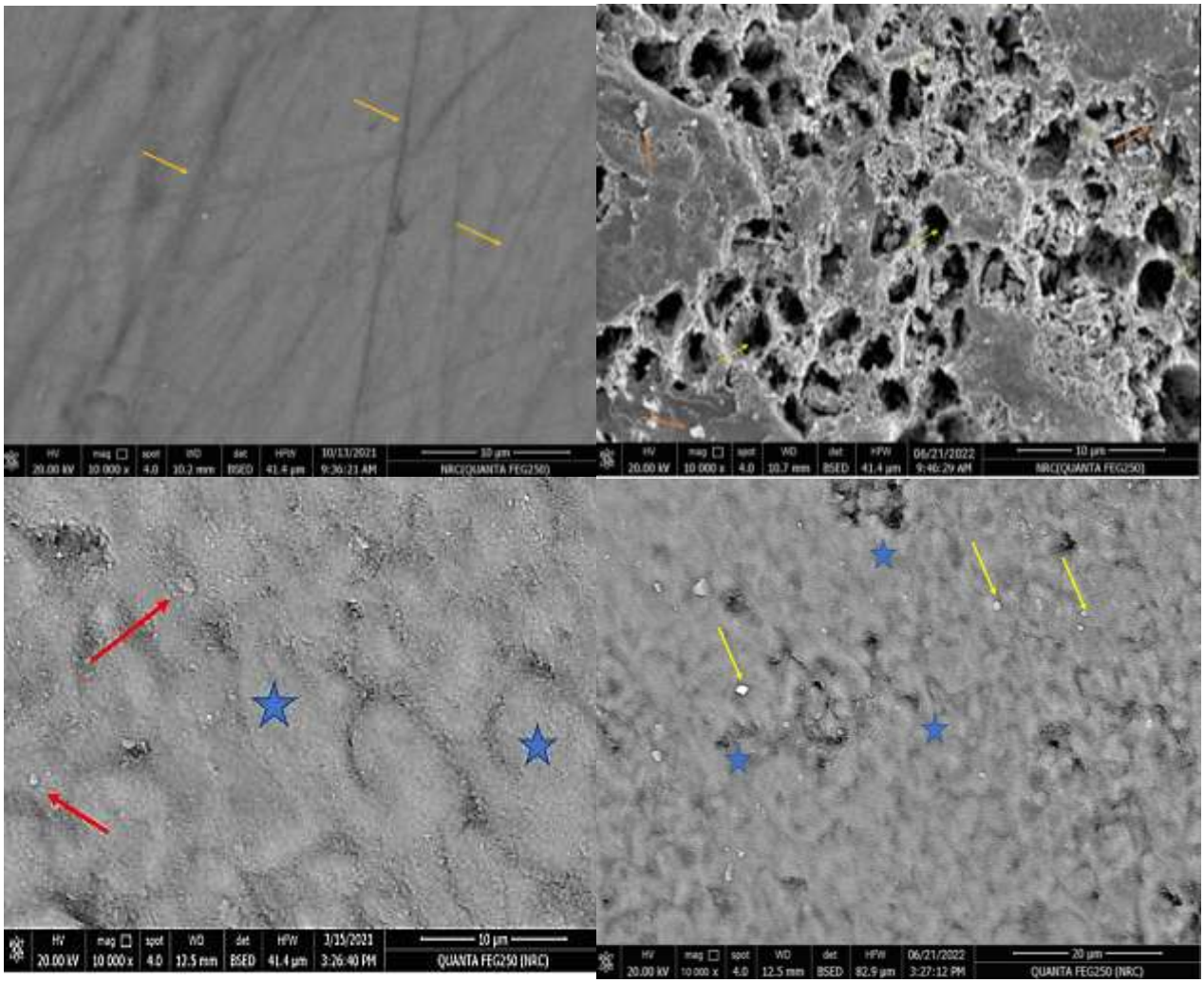


Figure 2: Showing scanning of deciduous teeth of all groups, GA (-ve control): smooth surface and countable number of surface scratches (yellow arrows). GB (demineralized):) showing large diameter micropores rod end appearance and interprismatic area (yellow arrows). White mineral precipitations (orange arrows). GC (prophylactic): Showing an extra layer of minerals precipitated at the surface (red arrow). No deep pitting defects or surface irregularities or any other surface defect. GD (treated): showing extra layer of minerals precipitated on the surface enamel (yellow arrows), only countable number of craters were found (asterisks).

maximal elevation of Ca/P ratio from **1.09 to 1.4**, and finally Group D which showed the mean Ca/P ratio increased from **1.09 to 1.23** as shown in figure 4. The f-ratio value is 10.7. The p-value is 0.01. The result is significant at $p < .05$.

Discussion

Loss of dental structure might occur in a variety of forms, including abrasion, attrition, abfraction, de-mastication, fine cracks, and resorption. Dental erosion is a chemical process where the hard surface is dissolved by acidic processes, and the microorganisms do not interfere with it *(Ispas et al., 2016).* After eruption, acids dissolution affecting tooth minerals: decay, erosion, and acid etching used for retention of restorations *(Fejerskov et al., 2003).*

Wang et al., 2014 and Lazzaris et al., 2015 illustrated that with evaluation of erosive potential of soft drinks on human enamel. The pH values of the soft drinks were below the critical pH value and low pH value causes more surface enamel loss. PH, titration acidity, and calcium concentrations are the most important in determining the potential of erosive materials. In the current study we used Popping candy as a demineralized agent.

In this study, we focused on the fact that when candies are kept inside the mouth, they melt and gas escapes which creates short feelings of entertainment and amusement, and that what children need (*Tabari et al., 2017*).

In the current study, we used forty samples divided into twenty permanent and twenty deciduous teeth. Teeth were randomly divided into eight groups, four groups for each type of dentition. Five samples each.: group A (-ve control group): no application of pop candies nor Theobromine, group B (+ve control group): application of pop candies only, group C (experimental group): application of Theobromine before and after emersion in pop-candies solution, and group D (experimental group): application of Theobromine after emersion in pop candies solution.

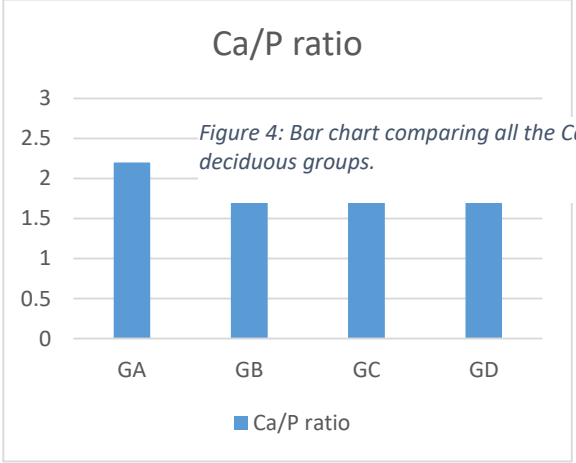


Figure 4: Bar chart comparing all the Ca/P ratios of all deciduous groups.

In a study by Mudumba et al., 2014 and Davies et al., 2008. The erosive potential was examined by measuring pH and neutralizable acidity as well as the ability of erosion in permanent and primary enamel (using profilometry). They compared the Discussion 87 parameters with orange juice as the control group. It was concluded that all sour candies are erosive even some are more erosive than orange juice.

Figure 3: Bar chart comparing all the Ca/P ratios of all permanent groups.

It has been shown in our study that permanent enamel is more resistant to erosion compared to primary enamel due to differences in hard tissue structure and mineral content. This was in agreement with (*Haghgou et al., 2016; Zheng et al., 2017*) who reported that a significant difference between primary enamel and permanent enamel teeth after 15 days of orange juice consumption.

We conducted this study to evaluate the prophylactic effect of Theobromine on enamel demineralization induced by pop candies on extracted deciduous and permanent human teeth.

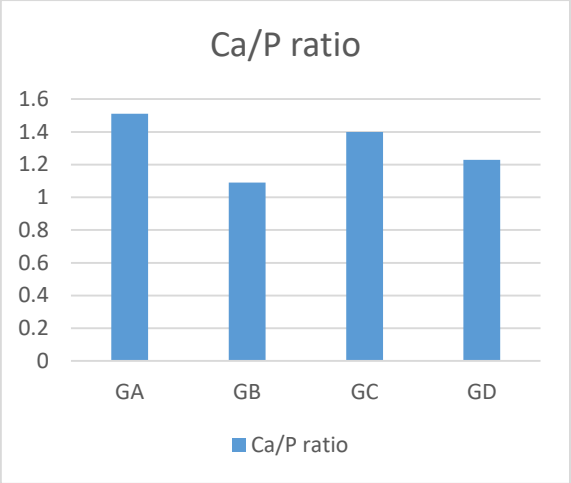
We evaluated the effect of Pop candies in permanent and primary teeth, and we found both groups affected significantly by demineralizing agent. Although the primary teeth were more affected than permanent teeth.

Tabari et al., 2017 had compared the effect of Kick Pop candies, strawberry Kick Pop candies, and Merdas popping chocolate and they concluded that orange Kick Pop candies reduce the microhardness of enamel and consequently lead to teeth erosion. This is in agreement with our results.

Our in-vitro study was in accordance with (*Taneja et al., 2019, Durhan et al., 2021*) where they analyzed the microhardness of the enamel surface values at the baseline and after treatment with theobromine. They showed the protective effect of theobromine treatment. Further investigations by *(Premnath et al., 2019)* using human teeth were conducted to evaluate how theobromine exposure modified the enamel surface in vitro by comparing theobromine dentifrices and fluoride containing dentifrices, the results of the in-vitro study have shown the ability of the theobromine to reverse the demineralization process.

Also, Taneja et al., 2019 investigated the remineralization potential of two concentrations of theobromine (100 mg/L and 200 mmg/L) with fluoridated dentifrice, NovaMin, and nanohydroxyapatite using DIAGNOdent, Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDXA) analysis. They concluded that Theobromine can be used as an effective novel remineralizing agent alternative to the already-available agents, as they found a significant increase in Ca/P ratio post application.

Our findings are in harmony with many studies such as Shawky and Khattab study which assessed the remineralizing potential of theobromine containing toothpastes compared to those with fluoride using scanning electron microscope. There was a significant difference found in calcium and phosphate levels between group theobromine and free group. In the same line, the results of EDXA analysis of *(Shawky and Khattab, 2021)* study indicated that theobromine was able to increase the minerals level more than fluoride, but the difference was not statistically significant.



Our study showed that theobromine caused calcium deposition in the experimental groups. Maximum deposition was in group C which has been treated with theobromine before and after immersion in pop candies solution. That came in agreement with *(Farhad et al., 2021)* who explained the mineral deposition caused by using theobromine as theobromine molecules are smaller than the micro-channels formed after acid dissolution of enamel; thus, they can pass through the enamel micro-channels and penetrate the hydroxyapatite crystalline structure. The higher electronegativity of oxygen and nitrogen in theobromine molecule ($C_7H_8N_4O_2$) attracts calcium (Ca) and phosphate ions which have low electronegativity and create new crystals of theobromine apatite [$Ca_{10}(PO_4)_6(OHC_7H_8N_4O_2)$].

Under normal conditions, Theobromine has the ability not only to stimulate enamel remineralization but also to protect it from

demineralization. Further in vivo investigations are needed to evaluate the effect of pop candies and theobromine inside the mouth.

Conclusion

We concluded that pop candies have demineralizing effect on both primary and permanent teeth. The effect of pop candies is different between two types of dentitions, and this may be due to difference in rod end direction and mineral content.

Theobromine as re-mineralizing agent helps in crystalline growth as in Group D (treated group) and it can increase thickness of enamel by adding extra mineralized layer as happened in Group C (prophylactic group).

Theobromine showed great effect on demineralization process by interrupting or by inhibition due to its ability to control mineral loss and this was confirmed by Ca/P ratio from EDXA and SEM of Group C (prophylactic group) in both types of dentitions.

Conflict of Interest

No conflict of interest.

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Ethics

This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo university on: 27-04-2021, approval number: 6421

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