EGYPTIAN
DENTAL JOURNAL
www.eda-egypt.org

VOL. 69, 1893:1904, JULY, 2023

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY

Submit Date : 14-03-2023 • Accept Date : 08-05-2023 • Available online: 25-06-2023 • DOI : 10.21608/EDJ.2023.194928.2453

COMPARISON BETWEEN SILVER DIAMINE FLUORIDE AND THEOBROMINE SOLUTIONS IN REMINERALIZATION OF DEMINERALIZED HUMAN ENAMEL [SCANNING ELECTRON MICROSCOPE AND EDEX STUDY]

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ABSTRACT

Introduction: By using fluoride, dental caries can be remineralized during the early stages of development. Although fluoride might be the first option for preventing cavities, too much fluoride exposure, particularly in children, can have negative effects, so we need alternatives. Due to their benefits, substances like theobromine and silver diamine fluoride are among the most hopeful ones for remineralizing demineralized enamel.

Purpose: To compare between solution forms of theobromine and silver diamine fluoride in the remineralization potential of the demineralized human enamel.

Materials & Methods: Twenty sounds mandibular first premolar were divided into 4 equal groups (5 samples each): group I Control group (C), group II demineralization group (D) subjected to demineralizing solution then 5 days PH cycle. The remaining two groups were similar to demineralization group with addition of treatment material, theobromine solution (T group) group III and silver diamine fluoride solution (S group) group IV, The samples were investigated by scanning electron microscope (SEM) and energy dispersive x-ray analysis (EDXA).

Results: Demineralization group's enamel was porous, revealing underlying enamel rods with serious rod core defects. The enamel surface improved with the theobromine solution group, and silver diamine Fluoride solution also improved the enamel surface, but to a lesser extent than theobromine did. EDXA revealed that the calcium phosphorus ratio displayed a descending order: (C > T > S > D). the difference in the calcium to phosphorus ratio (CPR) of the studied groups.

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There was statistically significant difference in the mean calcium/phosphorus ratio between groups (p = 0.002). For pairwise comparisons, demineralized teeth group had lower mean CPR (2.1 ± 0.6) compared with control (6.35 ± 0.7. p=0.001), demineralized teeth treated with TB (6.21 ± 2.3, p = 0.005) and demineralized teeth treated with SDF group (6.04 ± 1.1, p = 0.011). Contrarily, insignificantly (p = 0.755 and 0.891) higher mean CPR was reported in control group compared with both demineralized teeth treated with TB and demineralized teeth treated with SDF group. As well, insignificantly (p = 0.861) higher mean CPR was reported in demineralized teeth treated with TB than demineralized teeth treated with SDF group.

Conclusion: Theobromine solution and silver diamine fluoride are more effective remineralizing agents. But silver diamine Fluoride solution less than theobromine

KEYWORDS: Theobromine, silver diamine fluoride, remineralization, SEM, EDXA.

INTRODUCTION

Dental caries is defined as a chronic bacterial disease of the hard "calcified" structure of the tooth, characterized by decalcification of the inorganic substance followed by the destruction of the organic substance of the tooth to form a cavity, it affects humans of all ages.[1, 2]

Dental caries is now understood to be the localized, ongoing deterioration of teeth caused by oral bacteria. The initial lesion of dental caries is caused by the acid created when dietary carbohydrates are fermented. Since the 19th century, when sucrose became a popular daily sweetener used by many people around the world, dental caries have become an increasingly common problem.[3]

Management of dental caries

Management of dental caries is a challenging process that started in the past with extraction followed by surgical treatment, the minimal invasive until the concept of remineralization. [4]

1. The surgical model (drill and fill)

In the surgical process using handpieces and burs is recommended for the preparation of the cavity. [5] The surgical model deals with the results of caries (cavities) not deal with the cause of caries so no treatment of dental caries is done, only the cavities are cleaned and restored. [6]

The later technique was used for the management of dental caries for many years but it has a lot of disadvantages as using of anesthesia, unpleasant sensation of the patient, high cost, time consumption, and also unnecessary loss of the sound tooth structure also difficult to deal with this technique in some cases such as uncooperative patient and child. [7]

2. Minimal invasive dentistry

Minimal invasive dentistry is the concept of the patient care that deals with the causes of the dental disease, not just the symptoms based on the biological solution in addition to purely restorative.[8]

The aim of minimally invasive dentistry is to prevent and control oral disease and also keep teeth healthy and functional for life and this includes early caries detection and risk assessment, repair rather than replacement, Minimally invasive surgical intervention and remineralization of demineralized enamel. [9]

Demineralization

Enamel demineralization under oral conditions is generally accepted to result from acid conditions produced by the metabolism of cariogenic microorganisms in dental plaque. However, the formation of a dental caries is more complex than a simple dissolution process of the hard tissue. The striking histological feature of the incipient lesion is the presence of a relatively sound surface layer of enamel overlying the demineralized zone, i.e., the bulk of the mineral loss in the early stages of demineralization occurs at some distance from the enamel surface. For this reason the term "subsurface lesion" is used to describe this stage of the process. Another term widely used in this connection is "white spot", which describes the appearance of the affected enamel surface.[10]

Remineralization

Remineralization of the carious lesion is the focus of modern dentistry. [11] the term "remineralization" can be used to describe the process of the mineral deposition so the removal of white spot lesions, and of the chalky appearance of etched enamel can be done through this process. By this definition, remineralization occurs not only during periods of neutral pH but also during caries development. [12]

Dentine remineralization is more difficult and ineffective than enamel remineralization because enamel lesions have remaining seed mineral crystals, but dentine lesions do not. [13]

The degree of saturation of oral fluids (saliva and plaque) controls the remineralization process, when remineralization becomes the dominant phase, leading to lesion healing. An increase in calcium or fluoride concentrations in the oral fluids would appear plausible to improve lesion remineralization. [14]

Remineralization of the carious lesion is the focus of modern dentistry. The benefits of remineralizing agents have been demonstrated in a variety of formulations. [11]

Fluoride

Fluoride is one of the tooth remineralization agents that has been shown to help prevention of caries. For many years, fluoride's caries-preventive actions were attributed primarily to a decrease the enamel solubility. [15, 16]

Fluoridation methods used in the community, such as water and salt fluoridation, are significant in the prevention of dental cavities. Salt fluoridation is proposed as an alternate community option when water fluoridation is not possible. [17]

Fluorosis can be caused by excessive fluoride exposure, especially in children. Although fluoride is helpful at protecting enamel, its safety has been questioned, necessitating the search for new alternatives.[18, 19]

Silver diamine fluoride

Silver diamine fluoride is a water-like liquid that combines fluoride's remineralizing properties with silver's antibacterial properties.[20] It is a promising therapeutic agent for treating caries lesions that is applied to teeth with active lesions by using micro brush in an effort to stop the disease's progression.[21]

1- Chemical composition

Silver diamine fluoride (38% $Ag\ (NH_3)2F$) is a topical agent comprised of 24.4-28.8% silver and 5.0-5.9%fluoride, at pH 10.4 and marketed as advantage caries arrest by elevate oral care.[22]

2- Mechanism of action

The squamous layer of silver protein conjugates develops after SDF is applied to a decayed surface, enhancing the surface's resistance to acid dissolution and enzymatic digestion. On the uncovered organic matrix, hydroxyapatite, fluorapatite, silver chloride, and metallic silver all form. While the lesion depth decreases, the treated lesion's mineral density and hardness rise.[23]

3- Advantages

Simple, non-invasive technique for caries management quick, easy low coast and the dental personnel felt comfortable performing the procedures.[21] Play a major role in Caries prevention and arrest.[24]

A performed study that test the effect of SDF on the bacterial activity in carious lesion . 32 human teeth were artificially demineralized and inoculated 16 with streptococcus mutans (main causative organism in dental carious) and 16 with actino musis naeslundii (highly associated with root carious) In each group SDF or water was introduced to 8 blocks. By evaluated the biofilm morphology and viability. biofilm counts were decreased in the SDF group than the control group, carious lesion surfaces were harder the use of SDF than other group .in the streptococcus mutans group calcium and phosphate weight percentage after SDF application were higher than after water application .[25]

Theobromine

Characteristics Theobromine is a white powder that can be made synthetically from (3-methyl-) uric acid. It is primarily produced from cocoa husks as a byproduct of the production of chocolate.[26]

Although it dissolves in dilutions of alkali hydroxides and mineral acids, it is only very barely soluble in water (1 g/ 2,000 ml), alcohol (1g/2,220ml 95%), and boiling water (1 g/150 ml).[27]

Diuretic, smooth muscle relaxant, cardiac stimulant, and vasodilator are all terms used to describe theobromine. It is a very weak Central Nervous System stimulant, unlike caffeine, and exhibits both pro- and anti-oxidant properties .[26]

Advantages

Sugar-containing confectionery and eating chocolate is inextricably linked to tooth caries (i.e. chocolate is seen as a cariogenic food) however, Theobromine and cocoa inhibit dental caries. [26]

According to a conducted research, theobromine exposure increased the enamel's surface microhardness in comparison to sodium fluoride and aided in surface recrystallization.[28]

The strong inhibition of the metabolic activity of anaerobic bacteria by fluoride in wastewater treatment may well prove to be another decisive factor for the promotion of theobromine- and polyphenol containing toothpaste in the near future. [29]

Theobromine and fluoride have been compared in numerous publications, but the findings are inconsistent. A 2013 research by Amaechi et al. suggested that theobromine might be a good substitute for dentifrices that include fluoride additions. Theobromine had a remineralization ability on enamel lesions similar to fluoride despite having a molar level 71 times lower than fluoride .[18]

However, theobromine and fluoride have similar effects on enamel surface and remineralization ability, according to Nakamoto et al., 2016 .[30] When compared to fluoride theobromine was preferred since it is safer due to its low toxicity. [31]

A full assessment of the capacity of different types of theobromine and fluoride to remineralize demineralized enamel is required to determine the best caries prevention strategy.

Energy Dispersive X-ray Analysis (EDXA)

EDXA can be used to assess overall skeletal changes that often occur with age by measuring bone mineral content (BMC) and bone mineral density (BMD). EDXA measurements can also be used to provide information on early gender and ethnic changes in the rate of bone accretion and to determine the age when skeletal accretion ceases and when peak bone mass occurs. This information can be used to implement effective and timely measures with the objective of maximizing peak bone mass. Such measures may include calcium supplementation, dietary fortification, or programs promoting dairy products and other calcium- and vitamin-D-rich foods. This information can also be used to assess the impact of factors such as diet or lifestyle on measures of bone status in various minority populations.[32] In our study we used EDXA to determined the amount of the calcium and phosphorus on the tooth surface.

MATERIALS AND METHODS

Sample selection and preparation

In the current research, 20 healthy first premolars recently extracted for periodontal reasons were selected in this study after the approval of the ethical committee of Minia University. Teeth were collected from the surgery department in Cairo University. Teeth were coated with a nail varnish except for middle area of the labial surface of the teeth. Teeth were divided into 4 equal groups, each with 5 samples, and inspected to rule out those with enamel abrasions, erosions, or decay on the buccal surfaces.

Group "C" under control: (5) Samples were kept in distilled water throughout the investigation.

The remaining 15 samples were given a demineralization protocol to start caries-like lesions, which was loosely based on earlier studies.[33, 34]; and involved immersing the samples in a solution containing 2.2 millimole (mM) calcium chloride, 50 mM acetic acid, and 2.2 mM sodium dihydrogen phosphate for four days. Potassium Hydroxide will be added to the solution to modify the PH to around 4.5.

After the start of caries-like lesions, the samples were put through a 5-day PH cycle and split into 4 equal groups based on the PH cycle's treatment.

Grouping of specimens

Group name	Demineralization	Ph cycle	Treatment	Sample number
Control (C)	NO	NO	NO	5
Demineralization (D)	YES	YES	NO	5
Theobromine (T)	YES	YES	Theobromine solution	5
Silver diamine fluoride (S)	YES	YES	Silver diamine fluoride solution	5

- **The PH cycle similar to that proposed by Argenta et al., 2003.** [35]

1. Treatment substance (SDF or theobromine) application for three minutes or less.
2. A three-hour immersion in the demineralizing fluid.
3. Treatment substance application (SDF or theobromine) for 3 minutes.
4. Continue to soak in the remineralizing solution until the start of the following session.

Remineralizing solution is made up of (1.5 mM calcium chloride, 0.9 mM sodium phosphate), with potassium hydroxide added to bring the pH level to 7.

Powder version of theobromine (3,7-Dimethylxanthine) was provided. Theobromine was dissolved at the stated concentration in deionized water to create the solution.

SEM examination & Energy Dispersive X ray Analysis (EDXA)

Specimen preparation for scanning electron microscopy:

The samples were carefully cleaned for 3 minutes under running water, allowed to air dry, and then mounted using repositionable adhesive on the SEM holder. Each tooth had its center third of the buccal surface inspected. Under 500x, 1000x, and 2000x magnifications, the collected teeth were inspected at 20 Kilovolts (KV) using the secondary electron LFD detector. The Quanta 250 FEG (Field Emission Gun) Scanning Electron Microscope model has an EDXA Unit (Energy Dispersive X-ray Analyses) connected, an accelerating voltage of 30 KV, a magnification range of 14x to 1000000, and a resolution of Gun.1n**.**

Statistical analysis

The current study was non-randomized clinical control trial (n-RCT) that included four teeth groups:

- Group [C] (n=4): Control group
- Group [D] (n=4): underwent demineralization using demineralizing solution [2.2 millimole (mM) Calcium Chloride, 50 mM Acetic Acid and 2.2 mM Sodium Dihydrogen Phosphate].
- Group [T] (n=4): demineralizd teeth treated with Theobromine.
- Group [S] (n=4): demineralizd teeth treated with Silver diamine fluoride.

RESULTS

This study was performed to compare between solution forms of theobromine and silver diamine fluoride in the possibility for remineralization of demineralized human enamel using.

Scanning Electron Microscopic results

The center third of the buccal surface enamel of the control group showed a regular, relatively smooth enamel surface free of erosive lesions or porosity. The absence of the distinctive rod cross-section on the flat surface suggests that it is the complete intact outer rodless layer. (Fig.1)

The demineralization group had severe surface roughness and scattered porosity almost everywhere (Fig. 2).

Theobromine group's enamel surface showed a smooth enamel surface in the majority of the areas (Fig. 3). While some areas with circular concavities revealed the rod ends. Sometimes there were deep holes of varying sizes (Fig. 4).

In some areas of the enamel of the silver diamine fluoride group, there were no pits or exposed enamel rods to be seen (Fig. 5), but in other regions, there were erosive defects of varying sizes that resulted in the look of rough-surfaced enamel areas (Fig.5). Several areas of enamel displayed numerous whitish particles, which are silver nanoparticles, dispersed throughout the surface. (Fig. 6)

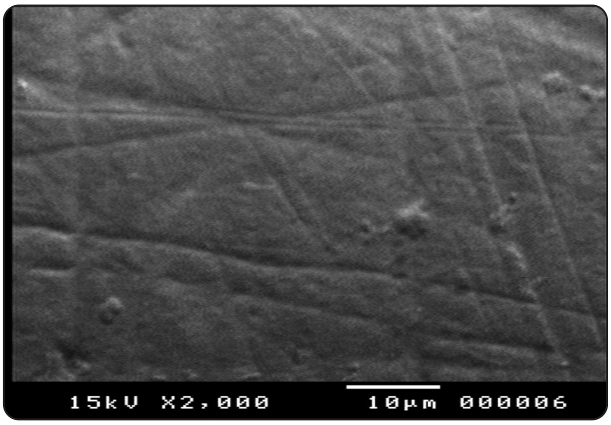

Fig. (1) Scanning electron micrographs of the control group showing relatively smooth enamel surface . [Magnification: A (x1000) B (x2000)]

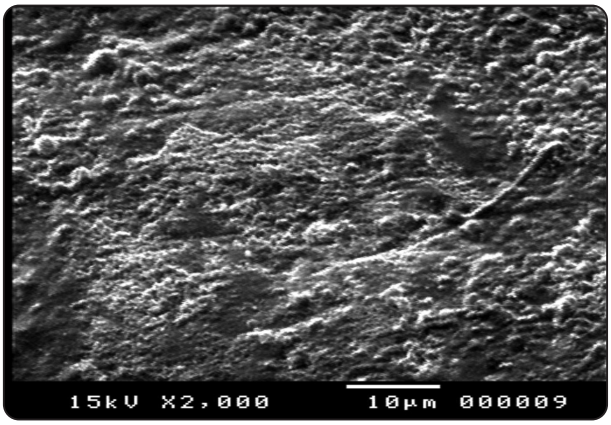

Fig. (2) Demineralization group. (A) and Porous enamel surface with severe roughness. [Magnification: A (x1000))]

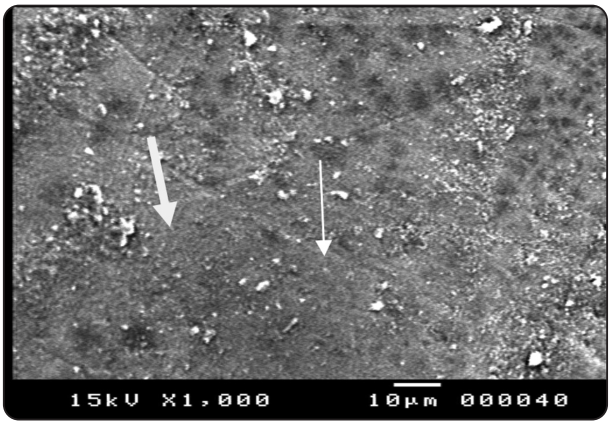

Fig. (3) Scanning electron micrographs of theobromine group demonstrated smooth enamel surface in most of the regions. [Magnification: (x1000)]

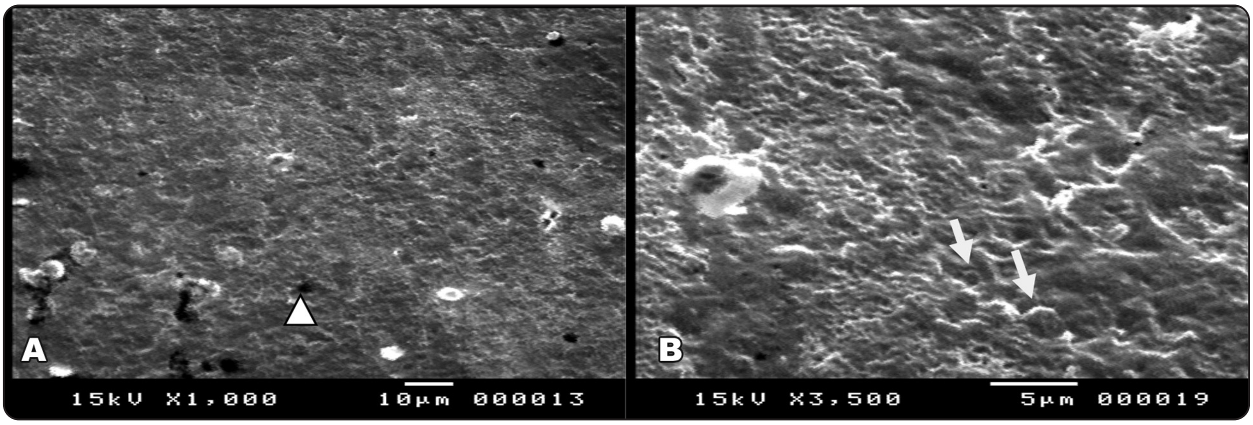

Fig. (4) Scanning electron micrographs of theobromine group. (A): The enamel rod end displayed shallow rounded dipping (arrow head). (b) the rod ends are exposed in other areas (arrow) [Magnification: A (x1000), b (3500)]

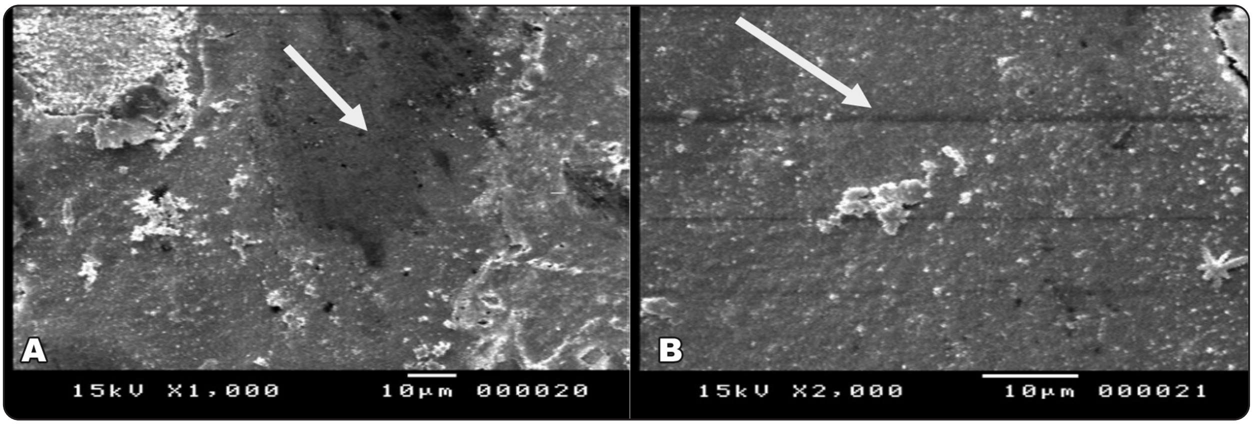

Fig. (5) Scanning electron micrographs of silver diamine fluoride group. (A) several regions of enamel showed erosive defects with variable sizes which lead to the appearance of rough-surfaced enamel areas (arrow). (B) Smooth enamel in some regions (arrow) [Magnification: A (x1000) B (x2000)]

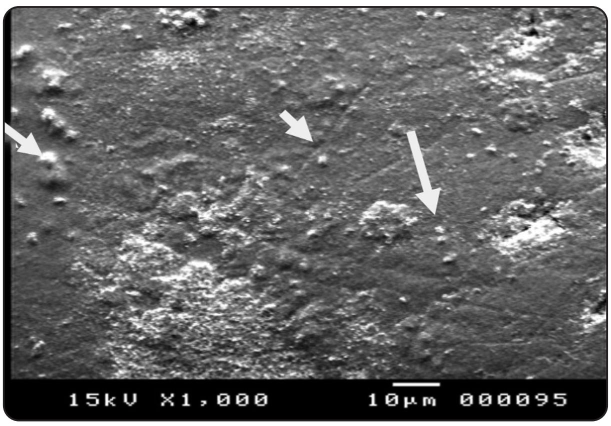

Fig. (6) Scanning electron micrographs of silver diamine fluoride group. (A), white particles are spread in the enamel surface that refers to silver nanoparticles (arrow) [Magnification: A (x1000)]

Energy Dispersive X-ray Analysis results

The current study was non-randomized clinical control trial (n-RCT) that included four teeth groups:

- Group [C] (n=5): Control group
- Group [D] (n=5): underwent demineralization using [2.2 millimole (mM) Calcium Chloride, 50 mM Acetic Acid and 2.2 mM Sodium Dihydrogen Phosphate]
- Group [T] (n=5): demineralized teeth treated with theobromine
- Group [S] (n=5): demineralized teeth treated with silver diamine fluoride

Following the measurement of the typical calcium and phosphorus percentages, the Ca/P ratio for each sample was computed. Calculations were made for the mean and standard deviation. The control group displayed the greatest mean value, while the demineralization group displayed the lowest mean value. The four groups are arranged in descending sequence as follows: control, theobromine, silver diamine fluoride, and demineralization (Table 2 and Fig 7).

Table 2 demonstrated the difference in the calcium to phosphorus ratio (CPR) of the studied groups. There was statistical significant difference in the mean calcium/phosphorus ratio between groups (p = 0.002). For pairwise comparisons, demineralized teeth group had lower mean CPR (2.1 ± 0.6) compared with control (6.35 ± 0.7. p=0.001), demineralized teeth treated with TB (6.21 ± 2.3, p = 0.005) and demineralized teeth treated with SDF group (6.04 ± 1.1, p = 0.011) (Fig. 7). Contrarily, insignificantly (p = 0.755 and 0.891) higher mean CPR was reported in control group compared with both demineralized teeth treated with TB and demineralized teeth treated with SDF group. As well, insignificantly (p = 0.861) higher mean CPR was reported in demineralized teeth treated with TB than demineralized teeth treated with SDF group.

TABLE (2): Comparison between the studied groups regarding Calcium/Phosphorous Ratio

	Group I (1) (n = 5)	**Group II (2) (n = 5)**	**Group III (3) (n = 5)**	**Group IV (4) (n = 5)**	**P-value**
• **Mean ± SD**	6.35 ± 0.7	2.11 ± 0.6	6.21 ± 2.3	6.04 ± 1.1	**= 0.002***
• **Median (R)**	6.4 (5.7 – 7)	2 (1.5 - 3)	6.3 (3.5 – 8.7)	5.8 (5.1 – 7.4)	
P-value**	**1 vs. 2 = 0.001**	**2 vs. 3 = 0.005**	3 vs. 4 = 0.861	1 vs. 4 = 0.891	
	1 vs. 3 = 0.755	**2 vs. 4 = 0.011**			

One-way ANOVA test was used to compare the mean difference between groups

*****Post-hoc test was used for pairwise comparison using Tukey's Correction***

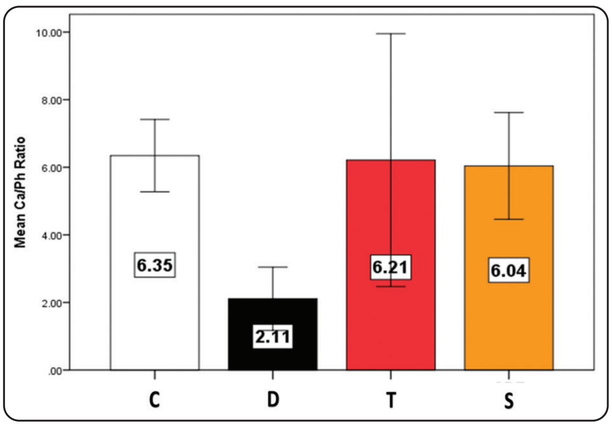

Fig. (7) Difference in Mean Ca/P Ratio between the studied group

DISCUSSION

An essential goal in the field of dentistry is the prevention of caries. The current research concentrated on remineralization as a powerful instrument for reversing an impending caries. Despite the fact that the literature has extensively addressed the enamel remineralization potential and its function in halting caries[36]. Both the classification of remineralizing materials and the methods for using these materials are up for debate.

In the present study we selected two remineralizing materials, the first is theobromine, a promising caries preventive material comparable to fluoride but safer than fluoride as theobromine is nontoxic[37]. The second material is sodium diamine fluoride a clear liquid that combines the antibacterial effects of silver and the remineralizing effects of fluoride and a promising therapeutic agent for managing caries lesions in young children and those with special care needs that have only recently become available in the United States.[38]

In the present study non carious human premolars were used for their wide labial surface area for both the creation of the artificial subsurface lesion taking into consideration the examination by using magnifying lens (TH-600600, China.) of ×7 to exclude any tooth with cracks or other structural defects. They were stored in distilled water.[39]

Chemical in vitro models were used in our research to initiate enamel caries-like lesions due to their simplicity, low expense, and experimental stability [40].

Since acetic acid could form detectable lesions even at pH 5.0 or higher, it was primarily used as a main component of demineralizing solution in the current study [41]. In the current study, we used PH cycling models to simulate the in vivo cyclical alternation of PH after the onset of caries-like lesions [41].

PH cycles could more accurately simulate the oral environment than conventional experimental methods. Additionally, the experiment's effective scientific supervision resulted in less variability [42].

In the samples that had been exposed to the acidic demineralizing solution, we found regions of erosive enamel. Similar organic acids like lactic acid and citric acid have also been noted in earlier research to predispose to erosion foci and regions of enamel surface dissolution [18, 43]. Additionally, we demonstrated a porous enamel surface following demineralization in our findings. According to Karlinsey et al. (2012), the administration of acid dissolves calcium and phosphate ions, causing gaps between crystals that result in enamel porosity[43].

In our study we used EDXA to determined the amount of the calcium and phosphorus on the tooth surface. The EDXA in our results confirmed the SEM findings because the demineralization group had a lower Ca/P ratio, which indicated a chemical demineralization process. This could be explained by a decrease in PH below a certain point, which causes the dissolution of enamel hydroxyapatite and subsequent demineralization [40].

In the current study, our findings showed that treated groups improved to varying degrees in terms of the features of the enamel surface. In terms of porosity, we found that the theobromine group was typically less advantageous than the silver diamine fluoride group. This could be explained by fluoride's dual role in inhibiting demineralization and preventing crystal disintegration [44] also improved remineralization through calcium ion attraction and binding to the surface of demineralized crystals [45]. Our findings are in line with those of Carrillo et al. (2018), who came to the conclusion that fluoride is the best therapy for remineralizing non-cavitated lesions.

In the current research, EDXA showed that the theobromine solution group had a better Ca/P ratio than the silver diamine fluoride group. Contrarily, Kargul et al. (2012) found that fluoride had a better effect than theobromine on raising the surface microhardness of demineralized enamel [37].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 silver diamine fluoride.

Although the later, Ca/P ratio of silver diamine fluoride group was near to the control group and this agreed with Favaro et al 2022 who conducted that the higher percentage of superficial microhardness alteration was verified on the surfaces treated with SDF [46].

Theobromine solution group's increase in the surface chemical composition of enamel corroborated Nakamoto et al's 2016 finding that theobromine caused the formation of larger hydroxyapatite crystallites in vitro [31]. Additionally, theobromine causes calcium and phosphate to combine into a crystal unit that is four times larger than hydroxyapatite, according to Sadeghpour et al's 2007 research[47].

Theobromine fluid increased the Ca/P ratio, according to our findings, but not to the same extent as the control group This coincides with Irawan et al2017's findings that theobromine improved enamel surface hardness after demineralization but did not restore it to its original hardness [16].

CONCLUSION

We came to the conclusion that all the tested materials had remineralization potential within the confines of the current research. A marginally more effective remineralizing agent than silver diamine fluoride is theobromine solution. In vivo research is required to verify this theory.

RECOMMENDATIONS

More in vivo studies are needed to evaluate the remineralization potential of theobromine and silver diamine fluoride of the demineralized human enamel and asses the appropriate material.

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