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COMPARATIVE EVALUATION OF THE EFFECTS OF SILVER DIAMINE FLUORIDE (COMMERCIAL AND LAB PREPARED) VERSUS NANO SILVER FLUORIDE ON DEMINERALIZED HUMAN ENAMEL SURFACES (IN VITRO STUDY)

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ABSTRACT

Background: - To evaluate and compare the effects of Silver diamine fluoride (commercial and lab prepared) versus nano silver fluoride on demineralized human enamel surfaces. Materials and methods: - A total 20 Extracted premolars for orthodontic reasons were collected from the Outpatient Clinic of surgical Department, Faculty of Dentistry, Suez Canal University. Each premolar was sectioned buccolingually into two halves, to obtain (40) specimens. These halves were randomly allocated into 5 groups according to different treatment modalities. **Group I** included 8 specimens were left untreated (negative control), **Group II** included 8 specimens were treated with demineralizing solution, **Group III** included 8 specimens were treated by Silver diamine fluoride (commercial form) after demineralizing solution, **Group IV** included 8 specimens were treated with Silver diamine fluoride (lab prepared) after demineralizing solution and **Group V** included 8 specimens that were treated with Nano silver fluoride after demineralizing solution. Each half was evaluated by Environmental scanning electron microscope and Energy dispersive x-ray spectroscopy .

Results: There were significant differences between groups at P-value (≤0.05) for Phosphrous, Calcium and Calcium/Phosphrous ratio except (Fluoide). The lowest value for elements was recorded in group (II) followed by control group and the highest value was recorded at group (V).

Conclusion: Silver Diamine Fluoride (lab made) is as efficient as commercial one in remineralizing demineralized enamel surfaces. Nano Silver Fluoride is more efficient than Silver Diamine Fluoride (lab made & commercial) on demineralized enamel surfaces.

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INTRODUCTION

Dental caries is an infectious microbiologic disease of the hard tissues. [1-3] It is a worldwide public health problem disturbing numerous communities.[4]

Dental caries develops from a cycle of demineralization and remineralization with several stages being either reversible or irreversible. It is started through demineralization of tooth hard tissue by organic acids created from fermentable carbohydrates by cariogenic bacteria that presents within dental plaque. [4]

Fluoride is the most commonly used remineralizing agent. As the pH of saliva rises, new and larger crystals that contain more fluoride (fluorapatite) formed, thereby reducing the enamel demineralization and enhancing remineralization.[5, 6]

Silver diamine fluoride (SDF, Ag(NH3)2F) has been proven to acquire antibacterial action that inhibits caries progression via stopping biofilm formation and helping enamel remineralization.[7] SDF has been established to be active for caries reduction in cavitated primary teeth and first permanent molar in 6-year old school children during a 36-month study. [7]

The anticariogenic effect of Silver diamine fluoride (SDF) [Ag (NH3)2F] is to releases fluoride ions quickly, therefore, assisting enamel remineralization. SDF is recommended by dentists to prevent the progress of caries mostly in Japan, Australia, and the United States. The product was cleared for sale in the United States in August 2014 by the Food and Drug Administration as a class II medical device.

SDF increases the resistance of demineralization, this occurs by formation of silver phosphate on enamel and enhancement of absorption of fluoride into dentin. This leads to blockage of dentinal tubules with further rises of minerals in peri and inter tubular dentin.[8]

Fluoride and silver in (SDF) interact with hydroxyapatite in tooth structure to form calcium fluoride and silver phosphate, which increase mineral density of enamel, increase the hardness of hydroxyapatite crystals, and decrease the depth of the carious cavity. [9, 10]

Today, Silver diamine fluoride can be prepared in lab to be available for arresting caries by simple, non-invasive, and low-cost medication.

Nano silver fluoride (NSF) is introduced as an alternative to silver diamine fluoride (SDF) in caries prevention, the main reason for that, is despite the ability of SDF in preventing and arresting caries e ven when applied once a year, it has some disadvantages such as black staining of carious tissue and metallic taste [11-13].

In vitro study was conducted on 2017 to determine the impact of Nano- Hydroxyapatite (NHAp), Nano Silver Fluoride (NSF) and Sodium Fluoride (NaF) varnishes on enamel remineralization, it was revealed that both NaF varnish and NHAp had similar effect in remineralizing initial enamel caries and that NSF have the greatest remineralization capability in comparison to the other two materials evaluated. [14, 15]

Until now From our knowledge's there is no study that evaluate and compare the effects of silver diamine fluoride commercial and lab prepared versus nano silver fluoride on demineralized human enamel surfaces. The null hypothesis was that there is no difference in remineralizing effect of these materials.

AIM OF THE STUDY

The aim of study was to evaluate and compare the effects of silver diamine fluoride (commercial and lab prepared) versus Nano sliver diamine fluoride on demineralized enamel surfaces (*in vitro*) by:

1- Environmental scanning electron microscope (ESEM).

2- Energy dispersive x-ray spectroscopy (EDX) for assessing minerals content .

MATERIALS AND METHODS

I -Materials

The materials and instruments were used in this study presented in table (1):

Ethics

The present study was approved from the Research Ethics Committee, Faculty of Dentistry, Suez Canal University code (205/2019).

TABLE (1): Materials brand names, composition and manufacturers:

Materials used in the study	Composition	Manufacturers
-Silver diamine fluoride (commercial form) concentration 44.800 p.p.m fluoride	Diamine Silver Fluoride 8% solution $Ag(NH_3)2F$	TEDEQUIM S.R.L Cordoba, Argentina
-Silver diamine fluoride (Synthetic form) concentration 44.800 p.p.m fluoride	Diamine Silver Fluoride 8% solution $Ag(NH_3)2F$	Generic at Department Of Chemistry, faculty Of Science,Suez Canal University
-Demineralizing solution	2.2 mM CaCl2, 2.2 mM NaH2PO4, and 50 mM acetic acid; 1 M KOH. pH 4.4.	Prepared in Organic Chemistry Departement, Faculty of science, Suez canal University
-Nano Silver Fluoride particles size (3.2–1.2 nm) (Synthetic)	• Chitosan (28,585 µg/ml); • Silver (376.5 µg/ml); • Sodium fluoride (5028.3 µg/ml)	Generic at Nanotech Egypt lab.
-Artificial saliva saliva	1.5 mmol/L Ca Cl2, 50mmol/L K Cl, 0.9 mmol/L K H2Po4 and mmol/L Tris buffer at pH of 7.3	Prepared in Organic Chemistry Departement , Faculty of science , Suez canal University
-Disc	SiC abrasive paper 800_2400(Silicon Carbide Grinding Disc abrasive paper)	Grit; struers, Copenhagen, Denmark

II -Methods:

II-1-Sample size calculation

Sample size calculation was performed using **G Power version 3.1.9.2** [16]

The effect size was 0.77 using alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power = 95%; the estimated minimum sample size (n) was a total of **40** samples for all groups (8 samples for each group).

II-2 -Sample collection

Twenty Extracted premolars for orthodontic reasons were collected from the Outpatient Clinic of surgical Department, Faculty of Dentistry, Suez Canal University with the following inclusion criteria:

- Sound surfaces.
- Free of white spots, cracks, and other defects.
- Not restored before.

Premolars were be stored in saline prior to preparation.

II-3 –Samples, Grouping and Adaptation:

- Each premolar was sectioned buccolingually into two halves, to obtain (40) specimens.
- They were divided into 5 groups.

Group I included 8 specimens that were left untreated and acted as a negative control group. Enamel surfaces in this group were remained intact. Specimens were stored in artificial saliva for 7 days at 37^o c in incubator

-The remaining specimens (32) were treated with demineralizing solution and divided into:-

Group II included 8 specimens that were treated with demineralizing solution for 1 hour three times a day, with 2- hour preservation in artificial saliva (pH 7) in between, this performed for 7 days at 37^o c in incubator. [17]

Group III included 8 specimens that were demineralized with demineralization procedures that were performed in group II. Then silver diamine fluoride (commercial) was applied with cotton swab for 2 minutes. Then specimens were washed with sufficient flow of deionized water for approximately 30 seconds. Finally they lightly dried with absorbent paper before being immersed in artificial saliva. They will be incubated at 37˚C (in an incubator) for 7 days in a tightly capped container filled with artificial saliva.[18]

Group IV included 8 specimens that were demineralized with demineralization procedures that were performed in group II. Then silver diamine fluoride (lab) was applied with cotton swab for 2 minutes. Then specimens were washed with sufficient flow of deionized water for approximately 30 seconds. Finally they lightly dried with absorbent paper before being immersed in artificial saliva. They will be incubated at 37˚C (in an incubator) for 7 days in a tightly capped container filled with artificial saliva.

Group V included 8 specimens that were demineralized with demineralization procedures that were performed in group II. Then Nano silver fluoride was applied with cotton swab for 2 minutes. Then specimens were washed with sufficient flow of deionized water for approximately 30 seconds. Finally they lightly dried with absorbent paper before being immersed in artificial saliva. They will be incubated at 37˚C (in an incubator) for 7 days in a tightly capped container filled with artificial saliva.[19]

II-4- Methods of Assessments:

1- Enviromental scanning electron microscope (ESEM):

All Samples of groups (I, II, III, IV and V) were taken out from artificial saliva for 2 hours to dry before analysis then, they were examined using environmental scanning electron microscope (QUANTA FEG-250) to observe ultra-morphology of enamel surfaces.

2- Energy dispersive x-ray spectroscopy (EDX):

Samples of groups (I, II, III, IV and V) were also subjected to energy dispersive x- ray spectroscopy to measure minerals content (calcium, Phosphorus and fluoride).

III- Statistical analysis:

All data was calculated, tabulated and statistically analyzed using suitable statistical tests as follow.

A normality test was done to check normal distribution of the sample, and all groups and must be showed normal distributions.

One way ANOVA was used to compare between the five groups for each element under study and Tukey test was used as post hoc to compare mean differences at significant level (P value). 0.05. Statistical analysis was performed using the computer program SPSS software for windows version 22.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels 0.05 (P- Value ≤0.5).

RESULTS

1- Enviromental scanning electron microscopic (ESEM) results:

- **Group I (negative control group):**

SEM evaluation of the negative control group revealed circumferentially arranged enamel rods (heads) with inter rod material (tails) producing a typical key hole appearance (**Fig. 1**).

- **Group II (demineralization group)**:

Examination of the specimens that were treated with demineralizing solution revealed well- defined apatitic-like crystals with intermittent interstices. The surface structure of enamel was porous, with larger inter crystalline spaces. Furthermore, demineralization increases the surface area and creates new sites in enamel. This was accompanied by partial loss of normal key hole enamel pattern. (**Fig. 2**).

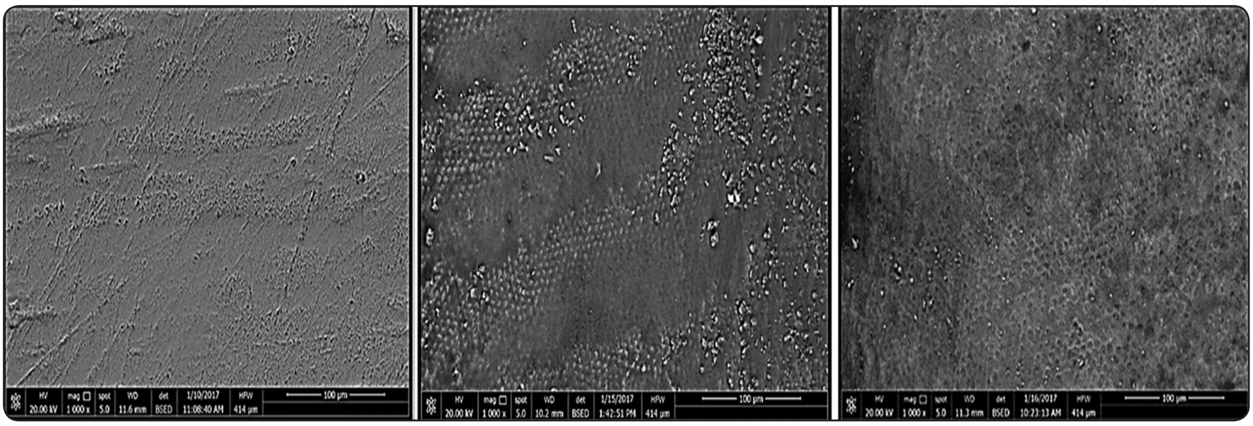

Fig. (1): SEM photographs of Group I (negative control) exhibited circumferentially arranged enamel rods and inter rod material with characteristic key hole pattern. (Magnification 1000X).

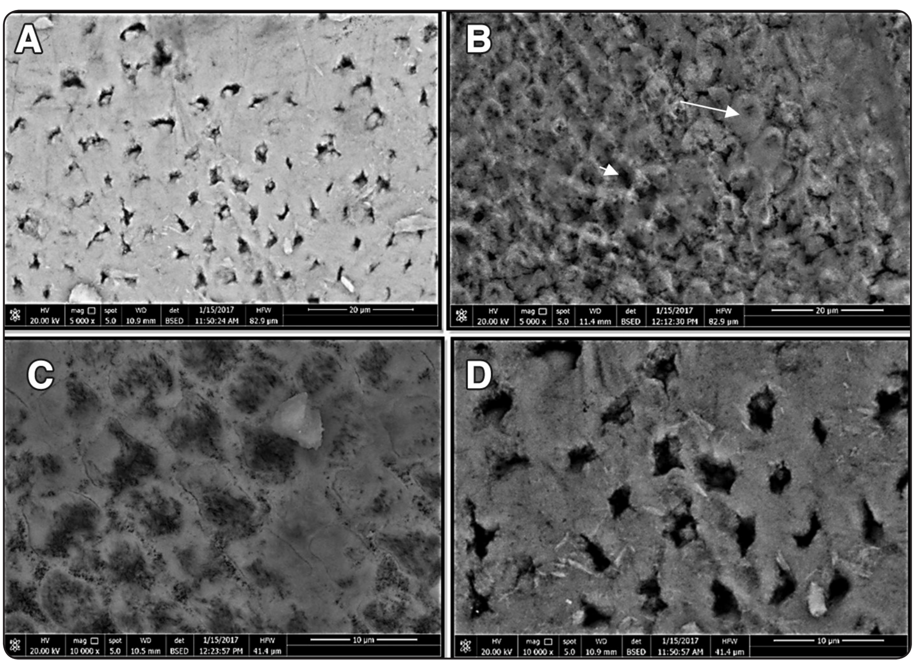

Fig. (**2**) (**A**), (**B**) SEM photographs of Group II revealing well- defined apatitic-like crystals (**arrow**) alternating with crevices (**arrow head**) (**magnification 5000X**). (**C**) (**D**) SEM photographs of Group II revealing higher magnifications of (**A, B**) (**magnification 10000X**).

- **Group III (commercial- form silver diamine fluoride group)**

The SEM images showed apparent decrease in number of gaps between crystals with formation of new small separate crystals distributed over the surface and partial regain of the characteristic key hole appearance when compared to group II (demineralization group) (**Fig. 3**).

- **Group IV (lab- made silver diamine fluoride group) :**

SEM examination of this group specimens revealed the incorporation of SDF particles into the fissures and crevices previously created by demineralizing solution and partial occlusion of these fissures (**Fig. 4**).

- **Group V (Nano silver fluoride) group:**

Specimens of this group characterized by regaining almost the normal architecture of the electron microscopic structure of enamel. By the obvious precipitation of NSF particles on the circumference of the enamel prism (prism sheath) together with the interprismatic substance, the typical key hole pattern was back again. (**Fig. 5**).

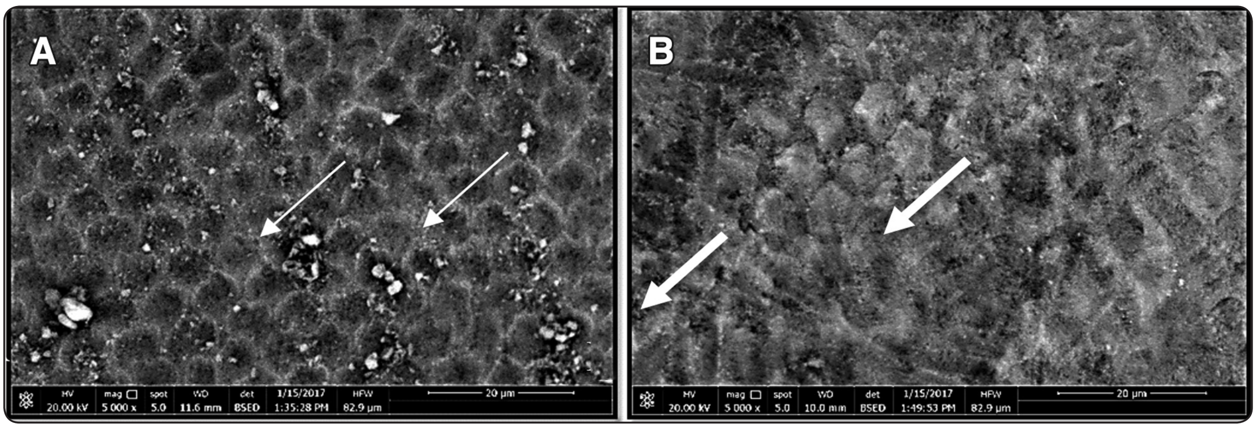

Fig. (**3**) (**A**) (**B**) SEM photographs of Group III revealing fewer number of gaps between crystals with formation of new small separate crystals (**arrows**) (**magnification 5000X**).

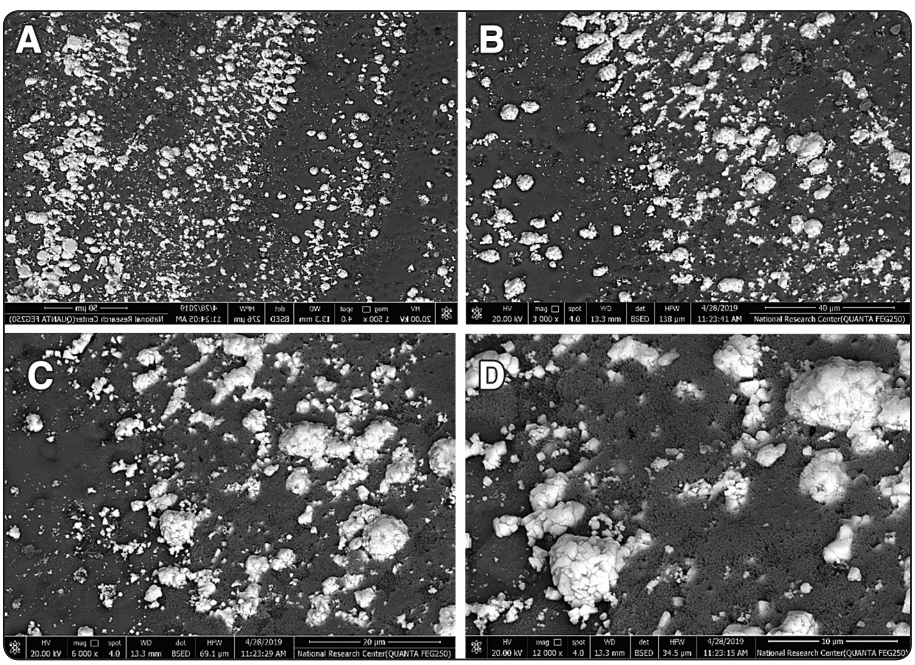

Fig. (**4**) (**A**), (**B**) SEM photographs of Group IV revealing SDF particles that are incorporated into the demineralized enamel surface (**magnification 1500X, 3000X respectively**). (**C**), (**D**) SEM photographs of Group IV showing higher magnifications of (**A**), (**B**) revealing the shape of SDF particles (**magnification 6000X, 12000X respectively**).

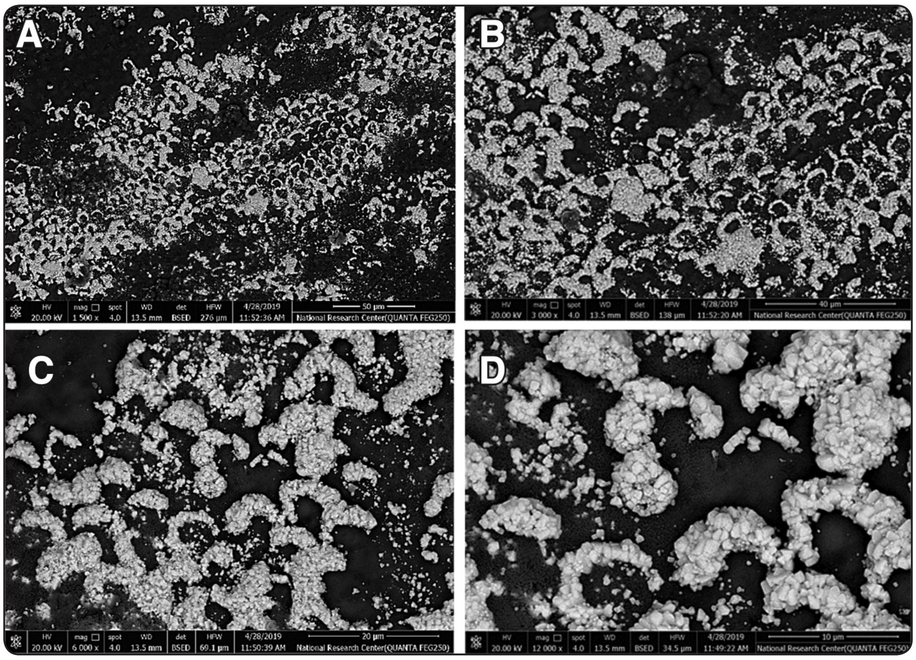

Fig. (**5**) (**A**), (**B**) SEM photographs of Group V revealing precipitation of NSF particles on the circumference of the enamel prism (prism sheath) (**magnification 1500X, 3000X respectively**). (**C**), (**D**) SEM photographs of Group V showing higher magnifications of (**A**), (**B**) revealing the shape of the typical key hole pattern (**magnification 6000X, 12000X respectively**).

2-Energy dispersive x-ray spectroscopy (EDX) results

The results in table 1, showed significant differences between groups at P-value 0.05 for **P**, **Ca** and **Ca/P** ratio except (**F**). The **lowest** value for **F** element was recorded in **group (II)** followed by **group (I)** and the **highest** value was recorded at **group (V)**. For (**P**) element the **highest** values were recorded in **group (V)** and **group (IV),** while the **lowest** value was recorded in **group (II)**. For (**Ca**) and the **highest** value was recorded in **group(V)**. **Ca/P** for all groups :- The **group(II)** is considered the **lowest group** for this ratio 1.83 followed by **Group(IV).**

Table (2): illustrates mean values for Calcium (Ca), Phosphorous (P), Fluoride (F) weights % and Calcium: Phosphorus ratio (Ca:P ratio) of the five groups

Table (2)					
Groups	**F**	**P**	**Ca**	**Ca/P**	**Ca/P%**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
Control	1.91±0. 60^{a}	14.00±0.69^{a}	31.30±2.24 ^{b}	2.24^{ab}	224.00^{ab}
Dem	1.27±0.10^{a}	7.66±3.37^{b}	12.83±1.11^{c}	1.83^{c}	183.00^{c}
SDF com made	2.28±0.85^{a}	13.89±2.16^{a}	28.89±2.6^{b}	2.09^{bc}	209.00^{bc}
SDF lab made	2.51±1.02^{a}	14.72±0.83^{a}	28.96 ±1.63^{b}	1.97^{bc}	197.00^{bc}
NSDF	2.95±0.54^{a}	14.94±2.19 ^{a}	35.41±3.50 ^{a}	2.41 ^{a}	241.00^{a}
P- value0.05	0.16 *ns*	0.000**	0.000**	0.000**	0.000**

*****, and different letters at the same column means significant difference between groups at P- value < 0.05.***

ns; means non-significant difference between groups at P-value 0.05

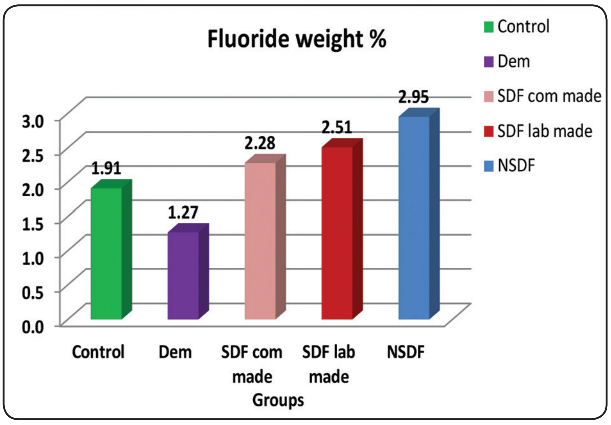

Fig. (6): Showing the mean values for Fluoride weight % of the five groups.

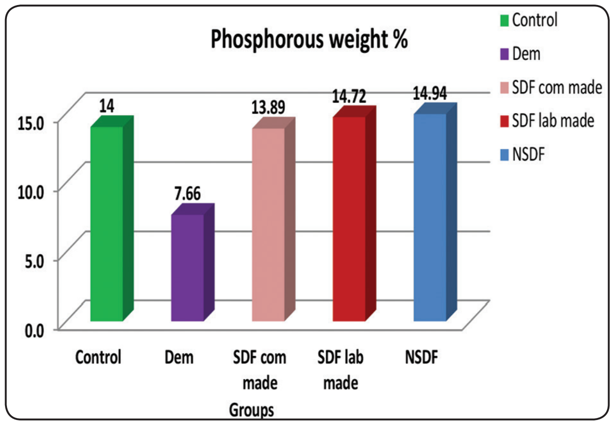

Fig. (7): Showing the mean values for Phosphorous weight % of the five groups.

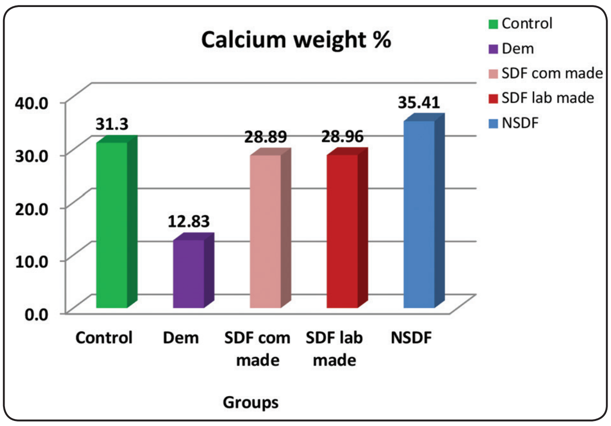

Fig. (8): Showing the mean values for Calcium weight % of the five groups.

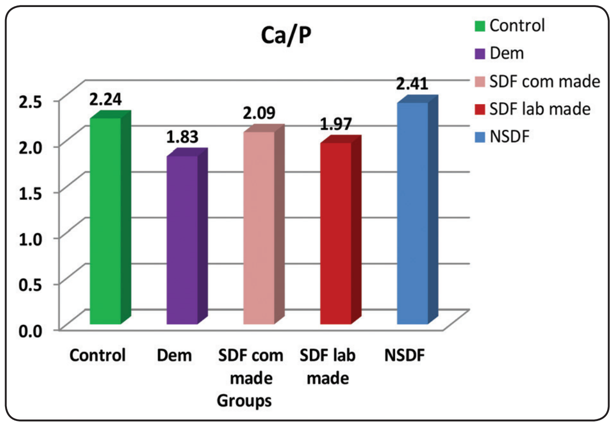

Fig. (9): Showing the mean values for Calcium/Phosphorus of the five groups.

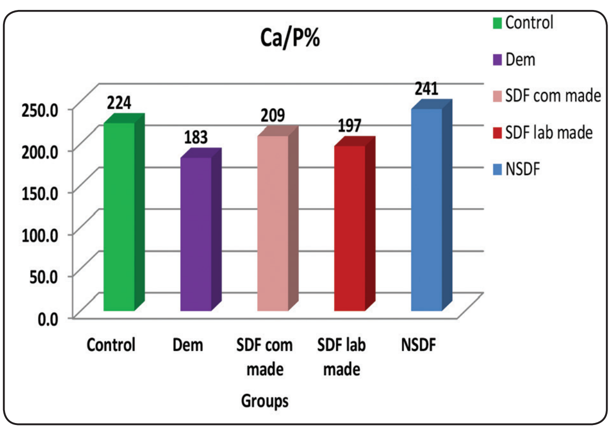

Fig. (10): Showing the mean values for Calcium/Phosphorus weight % of the five groups.

DISCUSSION

The present study was conducted to compare the effects of SDF (commercial & lab made) versus NSF on demineralized enamel surface. SDF is used to arrest dental caries in primary teeth and prevent pit and fissure caries in permanent molars accordance to Yamaga and Yokomizo [20].

Lab made SDF was prepared in the laboratory to reduce the purchase order from abroad and also to reduce the cost and time. No chemical or physical especially color and odour were observed for 12 months at room temperature .

NSF has been used due to its efficiency in stopping caries progression. [21]In addition use of silver nanoparticles in adhesives, composites, cements, and sealants inhibits biofilms and increase remineralization also, NSF was freshly prepared, had the advantage of not staining the dental tissue black, do not form oxides when reacts with oxygen in the medium and had no metallic taste as occurs in sodium fluoride treatment [22].

In this study, premolars have been freshly extracted from patients (12-14) years old for standardization and free from any aging changes that may present in enamel surfaces.All premolars were selected free from caries and any anatomical and morphological anomalies or structural abnormality such as enamel hypoplasia, amelogenesis imperfecta which can affect scanning Electron microscope results [23]. Specimens were stored in normal saline at room temperature to prevent dehydration.

PH cycling was performed to simulate caries challenge for 7 days this is in agreement with **Vieira et al** [17]. This disagrees with **Mohammadi and Farahmand**[23] who performed Demineralization cycle and repeated daily for 5 days then remineralization cycle for 2 days.

The null hypothesis was rejected, The results of scanning Electron microscope showed decrease in numbers of gaps with closure of fissures in NSDF treated group this is in accordance to **Zhao et al** [24] more than the two groups treated with SDF that promotes remineralization of hydroxyapatite of enamel.

The particles of NSF precipitated on the demineralized enamel with regaining of normal enamel architectures than SDF treated groups this may be due to small particles size (3.2–1.2 nm) which has high remineralization effects of Nano particles. On other hands, partial occlusion of fissures in demineralized enamel surfaces treated with SDF lab made more than SDF commercial made this is due to efficiency of lab made SDF on demineralized enamel surfaces.

The results of energy dispersive x-ray spectroscopy in this study revealed that the value of fluoride significantly increased in all groups treated with SDF and NSF treated group in comparison to demineralized group this attributed to high fluoride content in different treatments modalities groups that had remineralizing effects on demineralized enamel surfaces. This is in agreement with clinical trial which found that application of SDF and NSF had the same clinical efficiency in preventing dental caries [25]. This finding disagreed with Lee et al [26] who studied the effect of three different topical fluoride containing agents on incipient caries and they found that fluoride didn't affect surface micro hardness of enamel.

The value of phosphorus and calcium ions significantly increased in groups treated with lab made SDF and commercial SDF in comparison to control and demineralized groups this is accordance to an ex vivo study that reported hard remineralized layer rich with calcium and phosphate was performed after application of SDF [27].

Moreover NSF treated group showed significant increase in calcium and phosphorus ions in comparison to control and demineralized enamel groups this may be due to effects of Nano particles in increasing deposition of these ions on demineralized enamel surfaces ,that in accordance with **Ata Mostafa** [28] .

Calcium / Phosphorus ratio and Calcium – Phosphorus percentage significantly increased in NSF treated group in comparison to all treated groups this due to efficiency of small particles of Nano silver in remineralizing enamel surfaces[28].

CONCLUSION

1. SDF (lab made) is as efficient as commercial one in remineralizing enamel surfaces.
2. NSF is more efficient than SDF (lab made & commercial form) on demineralized enamel surfaces.
3. The possible mechanism of action for arresting enamel carries by either SDF or NSF is inhibition of mineral loss.

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RECOMMENDATIONS

1. SDF (lab made) can be used as efficient remineralizing agent.
2. NSDF can be used in arresting enamel surface caries both in vitro & in vivo.
3. Further studies should be conducted in vivo to study the effects of SDF & NSDF on demineralized enamel and dentin.

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