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Evaluation of Moringa Oleifera Extract on Demineralized Enamel of Primary Teeth

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ABSTRACT

Purpose: This invitro study was designed to evaluate the remineralization potential of Moringa Oleifera (M.O) extract in comparison with Casein PhosphoPeptide-Amorphous Calcium Phosphate (CPP-ACP) on enamel surface of human primary teeth using Environmental Scanning Electron Microscope (ESEM) &Analysing of mineral content. (EDAX)**. Material and Methods:** forty samples of extracted human teeth were obtained in accordance with guidelines from Al-Azhar University. EDAX & SEM were done for all the samples after demineralization, then they were divided into two main groups, the first group was subjected to CPP-ACP application for 5 minutes twice a day for a week. The second group was soaked in an aqueous extract of M.O for 5 minutes twice a day for a week. EDAX & SEM analyses were done for all specimens after remineralization. All data showed normal (parametric) distribution. Parametric data were presented as mean and standard deviation (SD) values. One-way Analysis of Variance (ANOVA) was used to compare between the two materials and de-mineralized enamel. The significance level was set at $P \leq 0.05$. **Results:** calcium& phosphorus levels of both groups of re-mineralization were higher than the demineralization level. Higher re-mineralization results were obtained from M. O extract. Conclusion**:** Moringa oleifera extract has a protective effect on enamel remineralization better than CPP-ACP.

KEYWORDS

Remineralization, Moringa extract, CPP-ACP, primary enamel.

INTRODUCTION

Several researches have been discussing enamel remineralization for decades, and they have come to the conclusion that "the non-invasive treatment" of early carilesions by remineralization was likely

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to be a great progression in the management of the disease clinically[1]. Choosing to remineralize hard dental tissues is the magical solution that ends the battle between prevention and surgical procedures in clinical dentistry [2].

One of the materials that have been studied for its effect on demineralized enamel, is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [3]. The application of Tooth Mousse containing (CPP-ACP) was found to reduce the softening of enamel surfaces and decrease the morphological changes that used to happen when the enamel was subjected to erosion [4].

The mechanism of action of CPP-ACP that prohibits enamel erosion is still quite undistinguishable, but there has been a suggestion that CPP-ACP can form micelles on the tooth surface, and these micelles might restrict the acid reaction and inhibit calcium loss [4]. However, these researches postulate that CPP-ACP can produce the effect of inhibiting erosion only when demineralization due to erosion has already occurred [5].

Almost 80% of the populations of the developing countries were found to completely depend on traditional medicines, mostly herbal drugs, for their primary health care requirements, according to the World Health Organization (WHO)[6]. Moringa oleifera is the most nutritious plant discovered till the present moment and has been making improvements in less-developed societies for ages. Several nutritional researches have been done to study this plant since the 1970s. It was found to have a rich and infrequent combination of nutrients, amino acids, vitamins and antioxidants, which enhance several beneficial properties such as anti-aging, anti-microbial and anti-inflammatory properties, sometimes it's called "Mother's Best Friend" and 'Miracle Tree' [7-8]

In 1992, Moringa Oleifera was approved by the (WHO) to be promoted into an alternative of imported food supplies to treat malnutrition [9]. The leaves and stems in particular are known to have a large amount of their calcium bound in calcium oxalate crystals. They also have more vitamins, iron and potassium than that found in common fruit & vegetables like carrots, spinach and bananas[10-11], in addition to compounds such as flavonoids, phenolics, and carotenoids which can be used as antioxidants [12-13].

Lately, there were some attempts to introduce flavonoids into the world of dentistry. Several flavonoids, such as proanthocyanidin & quercetin have been proven valuable in promoting oral health and have a remineralizing effect on artificial root caries [14]. Since Moringa Oleifera is proven to be rich in flavonoids, it's important to evaluate the remineralizing effect of Moringa Oleifera compared to CPP-ACP on primary enamel teeth.

MATERIAL AND METHODS

Materials used: (Fig. 1)

1. Phosphoric acid 37% gel.
2. Artificial Saliva. Prepared in department of biological chemistry Faulty of science, Al-Azhar University, Girls Branch.
3. Moringa Oleifera leaves extract. Obtained from Agriculture department. Moringa Unit in National Research Centre, Egypt (herbal store at NRC).
4. CPP-ACP (Tooth Mousse).

Teeth selection

Forty sound human primary teeth that were normally shed or extracted due to over retention was selected for this study. All teeth were obtained from patients whose guardians had already approved of using their biological sample, in accordance with guidelines from the ethical committee of Al-Azhar University. The samples belonged to healthy donors in average age of 7-12 years, had intact enamel surface with no pre-treatment of chemical agents nor any prior restoration [15]

Figure (1) Materials used in this study.

Exclusion criteria:

1. Teeth with cracks or discoloration in the area to be utilized for enamel sections.
2. Teeth with enamel hypoplasia or any other developmental anomalies.
3. Teeth obtained from a donor suffering from transmissible or systemic conditions affecting hard tissues. [16]

Sample preparation:

All teeth were rinsed thoroughly with running water & cleaned with soft brush after extraction to remove soft debris from the tooth surface. Teeth were cleaned and disinfected in 10% formalin for two weeks [17]. Roots of all teeth groups were cut to the level of cementoenamel junction using diamond disc. Cutting was carried out intermittently using water coolant. Then, polishing of enamel surfaces was done for 15 seconds using 3M soflex disc in a low speed and coolant. This was done to create a smooth surface. Then, rinsing of the specimens was carried out using distilled water, afterwards they were etched for 30 seconds using 37% phosphoric acid gel. Phosphoric acid was applied on the surface of interest then was washed for 30 seconds using water spray and then dried for another 30 seconds by the air spray.

Sample grouping:

After all teeth were demineralized, they were divided into two equal groups:

1. CPP-ACP group consists of 20 teeth specimens, that had CPP-ACP applied on their enamel surfaces for 5 minutes. Then CPP-ACP was removed with cotton tips and the specimens were stored in artificial saliva.
2. Moringa group consists of 20 teeth specimens that were etched then soaked into Moringa Oleifera solution and left for 5 minutes, then removed from the solution, cleaned with cotton tips and stored in artificial saliva.

The specimens of group 1 and 2 were subjected to the above-mentioned steps twice daily for one week. Afterwards, they were washed using distilled water and dried by air spray.

Preparation of aqueous extract of Moringa Oleifera:

The extraction, purification, and extract preparation were carried out in Agriculture department, Moringa Unit in National Research Centre, Egypt under supervision of Prof. Dr Abdullah Abul-Fotouh. Dried leaves of M.O were purchased from herbal store at NRC, Egypt.

Moringa leaves were air-dried under room temperature. One hundred grams of fresh leaves of Moringa oleifera Lam. were weighed out and crushed directly by grinder and dipped into 400 ml cold distilled water into a conical flask stoppered with rubber corks and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask and subjected to water bath evaporation where the aqueous solvent was evaporated at its boiling temperature of 100°C. The standard extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test [18].

Preparation of artificial saliva:

The artificial saliva used in this study was prepared in department of biological chemistry Faulty of science, Al-Azhar University, Girls Branch. The solution was prepared from analytical reagent grade according to Ringer's solution (type of artificial saliva). The solution typically contains 9g NaCl + 0.24g CaCl2 + 0.43g KCl +0.2g NaHCO3 all dissolved in 1 L of water [19]

Specimens' preparation for ESEM and EDAX:

The specimens were thoroughly rinsed with distilled water and dried with air spray for 30 seconds.

No further preparation was required. Samples didn't have to be coated with a thin layer of metal (usually gold or gold palladium), After completing the chemical analysis the same specimens were examined using the ESEM.

Environmental Scanning Electron Microscope (ESEM) analysis:

The treated enamel surface was scanned using ESEM and images (photomicrographs) of areas of interest were captured. The resulted images were analysed on Intel® Core13 based computer using Video Test Morphology® software (Russia) with a specific built-in routine for pixel intensity distribution and statistic.

The magnification used in this study was 2000 X & 5000 X. Enamel labial or lingual surfaces of the specimens were tested for surface roughness.

Energy Dispersive Analytic X-ray (EDAX) analysis:

All samples were analysed to determine the calcium and phosphorous mineral contents and the changes in the levels of these elements in demineralization/remineralization state. The surfaces analysed by EDAX included only the enamel surface of the specimens of all the groups. The area specified to receive EDAX irradiation was standardized for all specimens.

Each sample was placed at the analysing chamber in the ESEM, and then by using the ESEM we specified certain areas to be analysed. After irradiation with the EDX device, an EDX graph and a concentration table were presented by the software of the ESEM.

Statistical analysis:

One-way Analysis of Variance (ANOVA) test was used to compare between the two materials and de-mineralized enamel. For pair-wise comparisons, Bonferroni's post-hoc test was applied. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Scanning electron microscope (SEM) photomicrographs of demineralized enamel tooth surface

In all groups, the enamel specimens were exposed to etching & showed demineralization & erosion of enamel surface represented by areas of porosities & irregularities. (Fig. 2)

Scanning electron microscope (SEM) photomicrographs of remineralized enamel tooth surfaces:

Group 1: CPP-ACP group

Enamel surface treated with CPP-ACP showed partial restoration of the surface structure of the enamel which can be detected by significant loss of dissolution areas with minimizing of porosity depth & size which indicate remineralization. (Fig.2)

Group (2): Moringa leaf extract group:

Enamel surface treated with Moringa leaf extract revealed evidence of repair of surface structure, as observed by disappearance of dissolution areas, smooth & homogenous surface structure, presence of calcification deposits and remarkable lines of remineralization in all of the specimens. (Fig. 2)

Energy-dispersive X-ray spectroscopy (EDX) calculation:

Energy Dispersive X-ray analysis was done to find out calcium and phosphorus content in demineralized and re-mineralized enamel in each group as shown in (Table:1).

The table showed the mean, standard deviation (SD) and probability (P) value of phosphorus (P) and calcium (Ca) weight % and the Ca/P ratio in demineralized enamel and the remineralized groups: CPP/ACP group & Moringa group respectively. There was a significant difference between Ca & P weight % of different materials and de-mineralized enamel. Moringa showed the highest value of mean Ca & P weight %, CPP-ACP showed lower mean value, while De-mineralized enamel showed the lowest mean Ca & P weight %.

As for Ca:P ratio, Pair-wise comparisons showed that the two materials had no significant difference between each other. They both showed the highest mean of Ca:P ratios. De-mineralized enamel presented the lowest mean Ca:P ratio. Table (1).

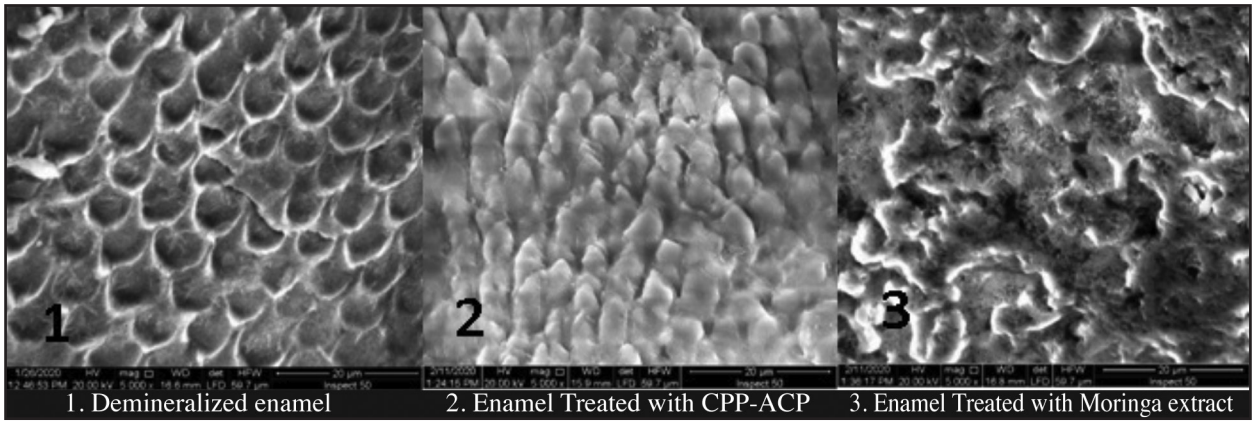

Figure (2) SEM photomicrograph (x5000) 1- (demineralized enamel): showing enamel with type II etching pattern and greater prism core dissolution giving the enamel a honeycomb pattern. 2- (CPP-ACP group): showing insufficient enamel surface remineralization as adherent granules or globules after the treatment by CPP-ACP. 3- (moringa group): showing increase mineral deposits that were scattered along the porous enamel surface after the treatment with moringa oleifera with apparent presence of remineralization lines.

Table (1): *Descriptive statistics for Ca, P, Ca:P ratio in all groups*

Mineral analysis	De-mineralized enamel		CPP-ACP group		Moringa group		*P*-value	Effect size *(Eta squared)*
	Mean	SD	Mean	SD	Mean	SD		
Ca weight%	28.4 ^{C}	7.7	33.5 ^{B}	4.5	39.8 ^{A}	5.6	<0.001	0.383
P weight %	14.1 ^{B}	2.3	13 ^{B}	3	15.8 ^{A}	2.6	0.006	
Ca:P ratio	2.03 B	0.48	2.68 A	0.56	2.55 A	0.31	<0.001	0.281

**: Significant at P ≤ 0.05, Different superscripts are statistically significantly different*

DISCUSSION

Primary enamel has its unique morphology that differs from permanent enamel. For instance, it has less minerals than in permanent enamel but higher coefficient of diffusion over it. General mineral density was lower in the outmost layers of primary enamel surface but showed no substantial differences close to the enamelo-dentinal junction[20].

On the other hand, it's difficult to depend only on fluoride for remineralization of primary teeth. Over-the-counter toothpastes contain fluoride that ranges from 1000 to 1500 μg/g concentration for adults, and from 250 to 500 μg/g concentration for children[21]. Thus, our study evaluated & discussed other materials that can be more effective & less harmful than fluoride in early caries prevention & white spot lesion remineralization.

This study aimed to evaluate the remineralization potential of Moringa Oleifera extract in comparison with CPP-ACP (Casein PhosphoPeptide-Amorphous Calcium Phosphate) on demineralized enamel of human primary teeth using: Environmental Scanning Electron Microscope (ESEM) and Analysing of mineral content (EDX), Ca and P were the elements of interest.

In this study, forty anterior primary teeth were collected for the trial. They were fixed with 10% formalin for two weeks before the trial, as recommended by the Centre of Disease Control and Prevention (CDC) [22]. Then teeth were etched using 37% phosphoric acid to produce a demineralized surface that simulates the morphologic changes which occur on the enamel surface when early attacks of caries take place [23]

CPP-ACP complex enhances remineralization by supplying the enamel surface with calcium & phosphate ions that boost the formation of hydroxyapatite crystals on the demineralized lesions [24]. Thus, it was only convenient to use CPP-ACP as a control group for this study.

The leaves and stems of M.O are known to have a large quantity of their calcium bound in calcium oxalate crystals. They also have more vitamins, iron and potassium than that found in common fruit & vegetables like carrots, spinach and bananas[25]. When involving herbs in oral care, usually the leaves are the most used part (25.44%) [26], so the design of the study included the leaves of M.O in particular to evaluate their effect on demineralized enamel.

The micromorphological analysis of Enamel in both groups after treated with 37% phosphoric acid showed etching pattern type 1, in which enamel prism core material undergoes dissolution but the periphery is intact giving the honeycomb appearance. Also type 2 and type 3 etching patterns were observed in few specimens in which the interprismatic substance undergoes dissolution and the core part of the rod is dissolved respectively.

The difference in etching patterns may be explained by the fact that enamel doesn't present a specific conditioning pattern, varying from tooth to tooth, surface to surface & in different areas of the same surface [27]

SEM analysis of CPP-ACP group showed decrease in surface porosities with appearance of mineralized deposits that were scattered along the porous defects.

Enamel prisms and interprismatic substance with microscopic surface irregularities were seen as granules or globules. This is due to mobilization of calcium and phosphate ions from CPP-ACP and repositioning on enamel surface. A previous study has documented this synergistic effect of CPP-ACP in remineralization [28].

The comparisons between CPP-ACP group and the demineralization results showed substantial decrease in surface roughness in the enamel surface and significant increase in Ca level. These findings confirmed that CPP-ACP has a protective effect against enamel demineralization.

A previous study also concluded this protective effect of CPP-ACP and explained it by describing CPP-ACP as s a reservoir of bioavailable Ca and PO_4 ions that are present at supersaturated levels and so reduce demineralization [29].

SEM analysis of specimens treated with Moringa Oleifera showed blocked enamel prisms with appearance of mineralized deposits along the porous defects. The comparisons between Moringa group and demineralization results revealed a significant decrease in surface roughness in the enamel surface and increase in Ca level.

The comparisons between CPP-ACP group and Moringa group showed significant change in surface roughness and calcium levels in enamel specimens. These results indicate that Moringa has higher remineralizing potential on enamel than CPP-ACP.

The ability of Moringa on increasing Ca levels was also discussed in a previous study that was conducted in oestrogen deficient conditions, where with ethanolic extract of Moringa Oleifera could effectively restore the reduced calcium level, correct the high rate of bone turnover and prevented further bone loss in rats. That indicated its potential in preventing osteoporosis as a natural way through herbal resource [30].

On the other hand, our findings come to a little disagreement with a previous study that concluded that there was no significant difference between Moringa Oleifera, and CPP-ACP paste regarding remineralization of enamel and dentin. However, it determined that Moringa had the same action of reducing erosion as CPP-ACP paste by localizing Ca and P ions at the tooth surface [25].

CONCLUSION

1. Moringa Oleifera extract has a high remineralizing potential on demineralized enamel of primary teeth.
2. Moringa Oleifera extract proved to have higher remineralizing effect than CPP-ACP on primary enamel teeth.
3. Application of CPP-ACP on primary demineralized enamel has a protective effect on remineralization.
4. Based on the EDAX data: Moringa has higher Ca & P weight % than CPP-ACP.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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