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Remineralization Potential of Casein PhosphoPeptide - Amorphous Calcium Phosphate in Comparison with Natural and Dried Milk: A Comparative Study.

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ABSTRACT:

Objective: The aims of the current study is to compare the remineralizing effect of a commercially present Casein PhosphoPeptide–Amorphous Calcium Phosphate (CPP–ACP in form of tooth mousse) in comparison with fresh buffalo milk and powdered milk (Nido) in terms of surface microhardness and surface roughness on experimentally induced enamel caries. **Methods:** Thirty upper premolar teeth extracted for orthodontic reasons, free from caries and defects were chosen, cleaned, roots of the teeth were separated and the teeth were poured in to special mold with the buccal surface exposed, that was polished with grit paper to obtain a flat surface. The teeth were randomly divided in to three groups (ten in each group). A profilometer was used to determine surface roughness, while Microhardness test of enamel was performed by Vickers microhardness tester. Readings for base line were obtained then the teeth were exposed to pH challenge (demineralization) and readings obtained, then the teeth were exposed to the experimental materials (CPP-ACP, buffalo milk and Nido).**Results:** A highly statistically significant difference between mean roughness and microhardness numbers for each stage and group of the materials in the study during baseline, demineralization and remineralization stages for both tests with the commercial CCP-ACP being superior in remineralization and although buffalo milk had less mean surface roughness than Nido, there was no significant difference between them, as for microhardness again CPP-ACP was superior, followed by buffalo milk and then the powdered milk, which indicate an anticariogenic activity occurs through direct contact with milk on tooth surfaces, as calcium, casein, and phosphate are incorporated into teeth,Dietary dairy recommendations provided by dental professionals to patients can be incorporated into patient education and caries prevention plans so that individuals exhibiting white spot lesions may benefit from counseling pertaining to dairy intake and use of CPP-ACP products to remineralize teeth.

Keywords: Milk, Nido, Remineralization, Casein phosphopeptide‒amorphous calcium phosphate.

I. INTRODUCTION

Dental caries is one of a series of chronic childhood diseases, having multifactorial etiology [1]. A series of strategies have been proposed for preventing caries, but no single strategy can be guaranteed to achieve maximum success as the disease is shifting through consequent periods of de and remineralization stages [2, 3].In the past fluoride was the corner stone and gold in preventing and treating caries, nowadays other novel materials are present and are trying to combat caries in other conservative ways [4].

Milk is an essential and excellent food for humans in all stages of life starting from birth onwards , and it has been shown to have anticariogenic properties in different studies[5, 6]. Derivatives from milk such as mixtures of casein phosphoproteins and amorphous calcium phosphate complexes have been demonstrated to exhibit anticariogenic effects in laboratory, animals and human in situ caries model experiments [7,8].

Buffalos are very popular in Iraq and in many parts of the world, they produce large quantities of fresh milk for consumption and to extract other milk derivatives[9,10] , while on the other hand, powdered milk is essential and present in every household in various commercial brands. Milk powder results from extraction of the water content present in milk. The main reason of converting milk into powder is to convert the perishable liquid raw material to a product that can be easily stored without substantial loss of quality, preferably a few years[11]. The aims of the current study is to compare the remineralizing effect of a commercially present Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP in form of GC Tooth Mousse) in comparison with fresh buffalo milk and powdered milk (Nido) in terms of

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surface microhardness and surface roughness on experimentally induced enamel caries.

II. MATERIALS AND METHODS

Thirty upper premolar teeth extracted for orthodontic reasons, free from caries and developmental defects were chosen , they were cleaned with non-fluoridated pumice and rubber cup with low speed hand piece, then the roots of the teeth were separated from the cemento-enamelleaving the crowns intact, molds made from a plastic tube with dimensions of 20mm diameter and 15mm depth were used to pour the teeth in to with cold cure acrylic with the buccal surfaces of crowns exposed , which were later ground and polished with grit paper (grit 400,600, 800) several times in a single direction to obtain a smooth and flat surface in order to perform the microhardness and surface roughness test [(12)].

A profilometer (Mitutoyo, Japan) was used to determine surface roughness, while Microhardness test of enamel was performed by Vickers microhardness tester (Wolpert, Germany), baseline readings of the teeth for both tests were recorded after randomly dividing the teeth in to three groups:

Group (1)= Ten teeth were subjected to pH cycling for ten days and then CPP-ACP tooth mousse was applied according to manufacturer instructions

As a layer of the cream was applied on the exposed buccal surface of each tooth twice daily and left for a period of 30 minutes, then washed off. This process was repeated daily for 7 days, then the teeth were put in deionized water until they were measured for microhardness and surface roughness [(13)].

Group (2)= Ten teeth followed the same protocol of demineralization as they were subjected to pH cycling procedure for ten days and then, the teeth were immersed in a quarter of a liter of raw fresh buffalo milk for 50 hours continuously, changing the milk every 2 hours [(14)].

Group (3)= Ten teeth same as the other groups were subjected to pH cycling of ten days, then the teeth were immersed in to a quarter of a liter Nido powdered milk after the addition of 4 tablespoonful of the powder to a cup water and mixing it thoroughly to prevent clumping, the milk was changed each 2 hours with new Nido powder milk that was freshly prepared. The remineralization treatment groups can be seen in figure (1).

For preparation of artificial enamel caries, the teeth were subjected to a pH cycling procedure for 10 days with a demineralizing solution which consisted of the following: Acetic acid 0.075 M/L, calcium chloride 1 mM/L, potassium phosphate 2 mM/L with 1 liter of deionized water adjusting the pH to4.3 for 6 hours, then the teeth were washed and each group of teeth was immersed in 250 ml of remineralizing solution for 17 hours [(15)].The remineralizing solution consisted of : Potassium chloride 150 mM/L, calcium nitrate 1.5 mM/L, potassium phosphate 0.9 mM/L with 1 liter of deionized water.

For the surface roughness, a profilometer with 50Xmagnification power,the parameter of maximum roughness height Rt which was obtained by obtaining the highest peak (Rp)and lowest valley (Rv) in the y direction along the central line of the area using the formula:(Rt=Rp+Rv) [(16)].Three measurements of surface roughness for each specimen was performed, and the average mean was considered for the study. For Microhardness test, Vickers hardness number was calculated according to the following formula:

VHN = 1.8544 (F/d^2)in Kgf/mm^2 (Mpa)

F: the indentation load (Kgf).

d : the diagonal of the indentation (mm) obtained from d=d1+d2/2 [(17)]

Three values of surface hardness for each specimen were obtained and the average was calculated and used in the analysis.

III. STATISTICAL ANALYSIS

Surface microhardness and roughnesswere reported as mean for each experimental group (at baseline, after demineralization and after remineralization)with the three different materials and statistically analysed by a one-way analysis of variance (ANOVA) and Duncan's multiple range test, results were considered significant when $p\leq0.05$ and highly significant difference when $p\leq 0.01$.

IV. RESULTS

Table(1)displays ANOVA analysis for surface roughness and microhardness for all the study groups, there was a highly statistically significant difference between mean roughness and microhardness numbers for each stage and group of the materials in the studyduring baseline, demineralization and remineralization stages.

Table (2) depicts means for the surface roughness and microhardness for all the groups, least surface roughness was observed at the baseline for the three treatment groups ranging between (0.490 - 0.560), that drastically increased to a range of (2.700-2.8900) during the demineralization stage, then increased to a rage between(1.130-1.460) for remineralization.

As for the microhardness values, highest values were observed at the baseline with mean values of 290.00 and 289.00, which later also declined rapidly to a range between (53.600-54.800). During the remineralization phase it increased to values between (123.700-73.900) being highest in the CPP-ACP group and lowest in the Nido group.

Table (3) demonstrate that there was a highly statistically significant difference in the remineralization stages of the three experimental groups of CPP-ACP, buffalo milk and Nido, while table (4) depicts that CPP-ACP mousse had the superior property for remineralization for surface roughness with a mean of (1.130) the less the values of surface roughness the better , and although the Nido powdered formula had the highest mean roughness (1.460)compared with pure fresh buffalo milk(1.330), no significant difference was observed between the two milk types. As for the surface microhardness, highest means was after the remineralization was again found in the CPP-ACP group followed by the buffalo milk and finally the Nido milk powder group with a statistically significant difference between them. Figures (2) and (3) displays the remineralization effect of CPP-ACP in comparison with fresh buffalo and dried powdered (Nido) milk in terms of surface roughness and microhardness.

V. DISCUSSION

Remineralization of teeth is a natural process of repair provided the teeth are not cavitated. The current in vitro study evaluated the effect of remineralization after an acidic challenge to teeth utilizing two types of popular milks that could be present in any household in Iraq which are buffalo milk, powdered milk(Nido) with a commercially available CCP-ACP, in the past fluoride was the gold standards and the key of remineralization[18], however other non fluoride remineralization materials have become popular and are being utilized, one of them is CPP-ACP[19].

The current study showed that for both surface roughness and microhardness tests there was a highly significant difference during the different stages of the experiment starting from base line to demineralization, after the pH challenge, and then after exposing the teeth to the experimental material during the remineralization stage, with each parameter in case of roughness and microhardness being opposite to one another, as the surface roughness was the least at base line for all the groups which increased dramatically after demineralization and had a significant less value after the remineralization stage with CPP-ACP being superior to both types of milk and although the buffalo milk showed less roughness in comparison with Nido, no significant difference was observed between the two milks. CPP-ACP is a specially formulated complex made up of phosphopeptide casein protein (CPP) derived from milk, it is structured by residues of phosphorylated serine and glutamic amino acid [20] it also contains amorphous calcium phosphate (ACP), whose ions are phosphorylated by the serine residues in CPP[21].Some studies found CPP-ACP is very efficient in inducing remineralization but slightly to a lesser extent than fluoride[22,23], while others found that a combination of fluoride and CPP-ACP can have a synergistic effect[24].The current study showed that CPP-ACP had superior properties than both types of milk for both tests, which might be attributed to the reason that it is a nanocomplex consisting of a cluster of amino acids that works by means of forming a of reservoir of calcium-phosphate that can be released that aid in remineralization[25].

Mankind has used milk from various animals as food since ancient times. Milk can be simply defined as a whitish liquid suspension and solution of many materials, it is a complex colloidal mixture of fat, proteins, lactose, and inorganic minerals[26], were there is an emulsification of fat globules and a suspension of casein micelles (which are made up of casein, calcium, and phosphorous found in an aqueous phase that contains lactose, proteins from whey, in addition to some salts [27].

Milk in composition is variable, basically containing four components in quantitative terms which are water, fat, protein and the sugar lactose while other components like minerals, vitamins, enzymes and dissolved gases are also present, information about the cariogenic or cariostatic potential of milk is limited.

The current study utilized two different types of milk including pure fresh buffalo and powdered Nido milk and both showed remineralization potential of different degrees with the buffalo milk showing superiority in increasing surface microhardness in comparison with powdered milk, this might be attributed to the reason that buffalo milk contains more protein, fat, lactose, amount of solid and nonfat solids in addition to that the buffering capacity of buffalo milk is greater than that of powdered cow milk [28], of course one should put in mind that there are certain factors that could vary in case of fresh milk like variations which are related to race and species of the animals from which the milk was obtained, and also other individual factors related to the health, nutrition of the animal, type of diet the animal consumes and its age. Another reason of buffalo milk being more superior in increasing remineralization can be related to is that it has more calcium than the powdered milk(10). Al-Ani and Al-Naimi(2020)(29) evaluated the remineralizing ability of commercial CPP-ACP with fresh buffalo and fresh cow milk in terms of surface roughness, and also buffalo milk proved to be superior to fresh raw cow milk, so this study was conducted to determine the effect of fresh buffalo milk in comparison with dried powdered milk, a previous study showed superior remineralization effect of different types of milk after adding commercial CPP-ACP to milk to remineralize enamel subsurface lesions using an established in situ model(30). The results of buffalo milk being superior to powdered Nido cow milk in remineralization can be attributed to the higher content of fatty acid for the buffalo milk in comparison with the Nido(31). Powdered milk also showed a lesser degree of remineralization, Powdered milk is, of course, cheaper than fresh milk. It's relatively non-perishable, in addition to being light in weight, and portable and can be stored for a considerable period of time in food stores and homes. In terms of increased surface roughness after the remineralization phace and not reaching the base line, rough surfaces of enamel result in increased susceptibility of the bacteria to attach to the tooth surface and therefor caries development, stain deposition increases also and the less surface roughness of teeth is always required.

In precious studies milk was blamed of being the etiological factor of developing early childhood caries or what is called "'baby bottle syndrome" (32,33,34).

The potential cariogenicity of any material depends largely on the manner and pattern of use. So milk can work in either direction, prevention or protection and parents of children should be health educated by dental practitioners about the cariostatic or cariogenic potential of milk, so that an infant does not go to sleep with a bottle containing any cariogenic material. In addition to actively discouraging the use of cariogenic liquids at bed time which can result in development of caries and have a deleterious effect on teeth.

VI. CONCLUSION:

Within the limits of the current study, CPP-ACP proved to be superior in remineralization, followed by pure fresh buffalo milk and finally powdered cow milk (Nido), all materials had some degree of mineralization that was not restored to the original baseline value, so focus on primary prevention is essential and mandatory to prevent early enamel caries

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Table (1) ANOVA For Surface roughness and Microhardness for the Study Groups For all the stages of the experiment

Type of Material		Sum of Squares	df	Mean Square	F	Sig.
CPP-ACP Roughness	Between Groups	29.865	2	14.932	513.596	.000**
	Within Groups	.785	27	.029		
	Total	30.650	29			
Nido Roughness	Between Groups	24.542	2	12.271	287.352	.000**
	Within Groups	1.153	27	.043		
	Total	25.695	29			
Buffalo Roughness	Between Groups	28.185	2	14.092	504.633	.000**
	Within Groups	.754	27	.028		
	Total	28.939	29			
CPP-ACP Microhardness	Between Groups	295654.867	2	147827.433	7.637E3	.000**
	Within Groups	522.600	27	19.356		
	Total	296177.467	29			
Buffalo Microhardness	Between Groups	315798.467	2	157899.233	6.522E3	.000**
	Within Groups	653.700	27	24.211		
	Total	316452.167	29			
Nido Microhardness	Between Groups	345184.267	2	172592.133	6.557E3	.000**
	Within Groups	710.700	27	26.322		
	Total	345894.967	29			

** Highly Statistically Significant P≤0.01.

Table (2) Mean Numbers for Surface Roughness and Microhardness for the Study Groups

Stage of Experiment		Roughness			Microhardness		
		CPP-ACP	Nido	Buffalo	CPP-ACP	Buffallo	Nido
Baseline	Mean	0.500 c	0.490 c	0.560 c	290.300 a	289.200 a	290.900 a
	N	10	10	10	10	10	10
	Std. Deviation	0.183	.179	0.171	6.832	7.525	8.198
Demineralization	Mean	2.860 a	2.700 a	2.8900a	53.600 c	54.800 c	54.100 c
	N	10	10	10	10	10	10
	Std. Deviation	0.171	0.258	0.179	1.955	3.155	2.514
Remineralization	Mean	1.130 b	1.460 b	1.330 b	123.700 b	93.500 b	73.900 b
	N	10	10	10	10	10	10
	Std. Deviation	0.157	.171	.149	2.751	2.461	2.331

Duncans Mutiple Range test ,means with different letters are statistically significant (Vertically)

Table (3)ANOVA For Surface Roughness and Surface MicroHardness for the Study Groups for the Remineralization Stage

Remineralization		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Roughness	Between Groups	0.553	2	.276	10.876	.000**
	Within Groups	0.686	27	.025		
	Total	1.239	29			
Microhardness	Between Groups	12587.467	2	6293.733	990.850	.000**
	Within Groups	171.500	27	6.352		
	Total	12758.967	29			

** Highly Statistically Significant P ≤0.01.

Table(4) Means and Standard Deviations for the Remineralization Stage for the Study Materials

Type of Material		**Roughness Remineralization**	**Microhardness Remineralization**
CPP-ACP	Mean	1.130 b	123.700 a
	N	10	10
	Std. Deviation	.157	2.751
Buffalo	Mean	1.330 a	93.500 b
	N	10	10
	Std. Deviation	.149	2.461
Nido	Mean	1.460 a	73.900 c
	N	10	10
	Std. Deviation	0.171	2.331

Duncans Multiple Range Test. Means with different letters are statistically insignificant P≤0.05.(Vertically).

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Figure (1) The Remineralization Treatment Groups. A-Commercial CPP-ACP Group B- Fresh Buffalo Milk Group. C- Nido Powder Milk Group

Figure (2) Mean Surface Roughness Values for the Test Materials after Remineralization

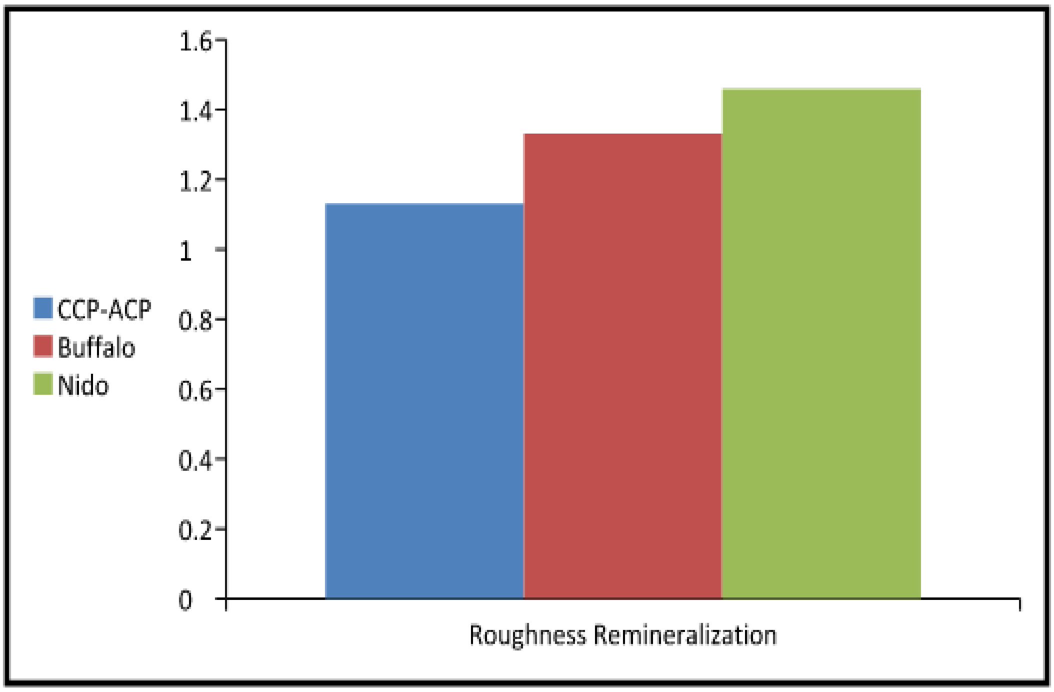

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Figure (2) Mean Micro hardness Values for the Test Materials after Remineralization