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EFFICACY OF CASEIN PHOSPHOPEPTIDE - AMORPHOUS CALCIUM PHOSPHATE IN COMBATING SPORTS DRINKS DENTAL ENAMEL EROSION – AN INVITRO STUDY

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ABSTRACT

Background:- Sports drinks are becoming increasingly popular as well as being encouraged with regular exercise. pH of these drinks are less than normal pH and found to be acidic. These acidic beverages are thought to increase potential for dental erosion. Casein Phosphopeptide - Amorphous Calcium Phosphate CPP-ACP complex helps to release Calcium, Phosphate ions to form apatite crystals. In acidic conditions, these calcium, phosphate ions are released from crystal complex and facilitate to reduce the extent of demineralization. This concept forms the basis for the study.

Aim:- To assess the efficacy of CPP-ACP in combating dental erosion caused by sports drinks.

Settings and design:- This study is an invitro clinical study.

Material and Methods:- Extracted premolar teeth of human dentition free of dental caries and hypo calcification were selected. Teeth were sectioned to obtain 3 enamel sections from each tooth to obtain 15 sections. Specimens were immersed in Sports drink (Red bull) Procedure (A),Sportsdrink (Redbull) +CPP-ACP Procedure (B) and Control Procedure (C) for 48 hours,24 hours,12 hours,6 hours,3 hours. pH Values before and after procedure were recorded and analyzed. After completion of immersion time, Tooth specimens were sectioned using Hard tissue Microtome; viewed for extent of demineralization under Polarized light microscope.

Statistical analysis:- One way ANOVA was used to compare the difference in mean of demineralization among the groups.

Results:- Specimens subjected to Sports drink(A),showed wider areas of enamel demineralization. One way analysis of variance (ANOVA) between the groups shows that F value is 4.977, df=2, at 5% level of significance. Sports drink+ CPP-ACP (B)(193+ 14) showed decreased amount of demineralization when compared to Sports drink (A)(264+19).

Conclusion:- CPP-ACP has the ability to reduce demineralization caused by sports drinks. It facilitates Protective action of Loss of mineral content of teeth caused by Sports drink erosion.

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INTRODUCTION

Sports drinks are originally created as Carbohydrate and electrolyte aqueous formulation to supplement performance and to prevent dehydration during strenuous exercises. The pH of these drinks are found to be acidic and less than normal pH (=7). Some of the Sports drinks include Coco cola, Diet coke, Gatorade, Redbull drinks. Sports drinks facilitate in enhancement of physical and mental performance of sports individuals. Excessive consumption of these acidic beverages thought to increase the potential of dental erosion. Dental erosion is defined as an irreversible loss of dental hard tissue due to a chemical process without involvement of microorganisms. (Imfeld, 1996) It may be caused by either extrinsic or intrinsic factors. One of the extrinsic causes of dental erosion is excessive consumption of acidic beverages. (Dugmore and Rock, 2004) The prevalence of erosion is thought to be increasing, reflecting the wide availability and frequent consumption of acid beverages, fruit juices, carbonated beverages, wines, sport drinks. (Barbour *et al.*,

2005; Ramalingam *et al*., 2005) Erosive potential of acidic drinks depends mainly on the acids contained and may result in decrease in pH of oral environment. As a result it causes acidic environment and loss of enamel structure. In the oral environment, tooth structure undergoes continuous process of demineralization and remineralization if this balance is interrupted, demineralization will lead to progressive deterioration of tooth structure. Preventive methods that may reduce and decrease the tooth demineralization include increased acid resistance of tooth structure and remineralization process require Calcium, Phosphate and Fluoride. CPP-ACP (Calcium phopsphopeptide, Amorphous calcium phosphate) is the newer product which is a derivative of milk. Calcium and Phosphate ions move out of CPP enter enamel rods and reform apatite crystals. Milk protein casein can be digested with trypsin and complexed with calcium and inorganic phosphate ions to produce CPP-ACP. Reynolds etal describes the mechanism of action of CPP-ACP that nanocomplexes are readily soluble in saliva that allows them to localize in supragingival plaque. CPP-ACP in plaque can enter lesion fluid as an intact complex and diffuse into the lesion. (Reynolds *et al*., 2003) Casein phosphopeptides (CPP) containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- have a remarkable ability to stabilize calcium phosphate (ACP) in metastable solution. (Cochrane and Reynolds, 2009) Cochrane *et al* states that recently, it was shown by immunolocalization that CPP were present inside a remineralized enamel subsurface lesion, indicating that they can navigate the size and charge impediments to enter the lesion. (Cochrane *et al*., 2010) The CPP-ACP nanocomplexes can be used to deliver high concentrations of bioavailable Calcium and Phosphate ions intraorally to inhibit demineralization and promote remineralization. (Reynolds, 2008) CPP-ACP releases Calcium and Phosphate ions into plaque fluid by equilibrium release, Competition release, pH difference and hydrolytic release. (Cochrane and Reynolds, 2009) Released calcium, phosphate ions in the plaque fluid will increase their activity and increase diffuse gradients of various ions into the lesion. CPP-ACP binding to apatite crystals face in the surface of lesion, keep the diffusion pathways open to allow ions penetrate deeply throughout the body than just the surface layer.

During an erosive attack, the CPP‑ACP could release Ca^{2+} and $PO4^{3}$ ions, supersaturating the media with these ions and creating an environment favorable to enamel remineralization. (Reynolds, 1998; Reynolds *et al*., 2008) Sports drinks often result in erosion of tooth surfaces with increase in pH and demineralising enamel lesions. In oral environment these calcium and phosphate ions released from CPP-ACP may combact the loss of minerals. The purpose of present study is to assess CPP-ACP efficacy in contrasting dental erosion caused by acidic nature of Sports drinks. Objective of the study is to estimate the pH values of Sport drink (Redbull) before and after immersion time and to estimate the loss of mineral content of the tooth exposed to sports drink with and without CPP-ACP.

MATERIALS AND METHODS

Selection of toothsamples

Extracted 32 premolar teeth which were free from hypocalcification, caries, and anyother initial enamel lesions were collected prior to start of study. These teeth were collected and stored in 5% formalin solution in sterile containers. These premolar teeth obtained were sectioned into three equal halves .Roots were cut and crowns were cleaned with pumice. A small window of 4x4mm dimension was prepared on the surface of sectioned teeth by coating all the surfaces with nail enamel varnish.

Categorizing tooth samples into procedure groups

Procedure-A

10ml of Sports drink (redbull) was carried into sterile containers with a graduated measuring cylinder. Sterile containers were divided according to immersion time of 48hrs, 24hrs, 12hrs, 6hrs, 3hrs as group-1, group-2,group-3,group-4,group-5. All these containers were labeled clearly with immersion time.(Figure-1)

Procedure-B

0.25% of CPP-ACP was diluted in 99.75% of Redbull drink. This solution of Redbull+CPP-ACP was taken in a sterile conical flask with cotton plug and complete dilution of the drink and CPP-ACP was obtained with orbital shaker (SCIGENICS BIOTECH) which was set at 125rpm (rotations per minute). Samples were divided into 5 groups, group-1,group-2,group-3,group-4,group-5, same as procedure-A based on immersion time of 48hrs,24hrs,12hrs,6hrs,3hrs.This forms procedure-B

Procedure-C

Same quantity of distilled water, 10ml was taken in the sterile containers with a graduated measuring cylinder. Sterile containers were divided according to immersion time of 48hrs, 24hrs, 12hrs, 6hrs, 3hrs as group-1, group-2,group-3,group-4,group-5. All these containers were labeled clearly with immersion time. Distilled water group forms the control group (procedure-C)

P^H analysis

The sectioned teeth were properly immersed into the 3 procedure groups.The surface which is left with a window space was subjected to sports drink and rest of the tooth not subjected as it was coated with nail enamel varnish. P^H values of the drink; drink+CPP-ACP and distilled water was assessed with the help of digital P^H meter (ELICO L_1 12O) before the start and end of the immersion time. The P^H values of the samples were obtained and illustrated in (Table-1).

Hard tissue microtome sectioning

After the immersion time, tooth samples were removed and the samples were subjected to hard tissue microtome sectioning (Figure-2). The tooth samples were mounted in the acrylic blocks and thin sections were made with hard tissue microtome (LEICA SP1600). Thin sections of tooth obtained from microtome were mounted on glass slides with coverslips. These microscopic slides were viewed under polarized light microscope (Prog Res cs), similarily under electron microscope.

Inspection of microscopic images

Polarized (Prog Res cs) microscope was connected to computer and microscopic images were installed into computer software.

Table-1:- pH Values of the samples in all the groups before and after completion of immersion time

Category	Immersion time		Procedure-A		Difference in pH values	Procedure-B		Difference in pH value	Procedure- c	
			before	after		before	after		before	after
Group-1	48hrs	pH	2.54	4.72	2.18	2.63	4.80	2.17	6.99	6.99
Group-2	24hrs	pH	2.53	4.35	1.82	2.62	4.48	1.86	6.99	6.99
Group-3	12hrs	pH	2.54	4.33	1.79	2.61	4.37	1.76	6.99	6.99
Group-4	6hrs	pH	2.55	3.36	0.81	2.60	3.39	0.79	6.99	6.99
Group-5	3hrs	pH	2.54	3.04	0.5	2.64	3.02	0.38	6.99	6.99

Table- 2 :- One way ANOVA of Loss of mineral content of the Samples

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Procedure A	5	264.2754	190.90107	85.37355	27.2404	501.3104	87.29	560.06
Procedure B	5	193.6920	141.37600	63.22527	18.1505	369.2335	81.71	435.25
Procedure C	5	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	15	152.6558	171.74629	44.34470	57.5459	247.7657	.00	560.06

	Sum of Squares	df*	Mean Square	F ¶	Sig. †
Between Groups	187233.490	2	93616.745	4.977	.027
Within Groups	225721.561	12	18810.130		
Total	412955.052	14			

* df= degrees of freedom, ¶= Fishers test value †=p<0.05

A=redbulldrink,B=redbull+CPP-ACP,C=Control

Figure 1. Picture depicts Samples categorized into 3 procedures with 5 groups based on immersion time

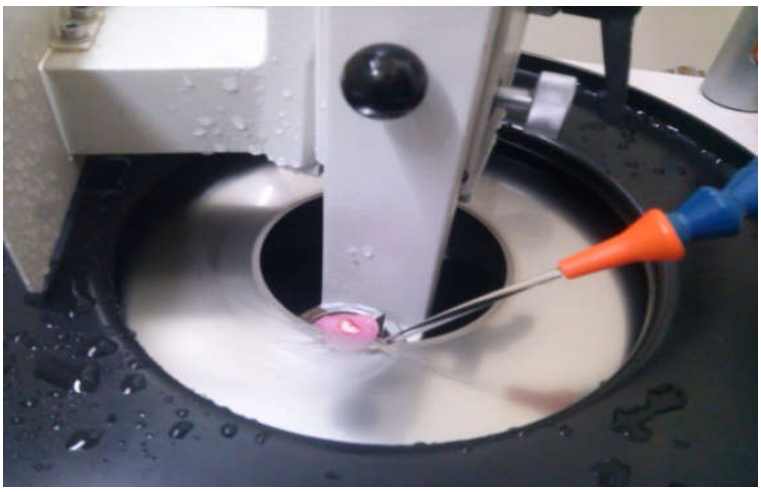

Figure 2. Hard tissue microtome sectioning of tooth samples

Figure 3. Microscopic images viewed under Polarised microscope at various immersion times

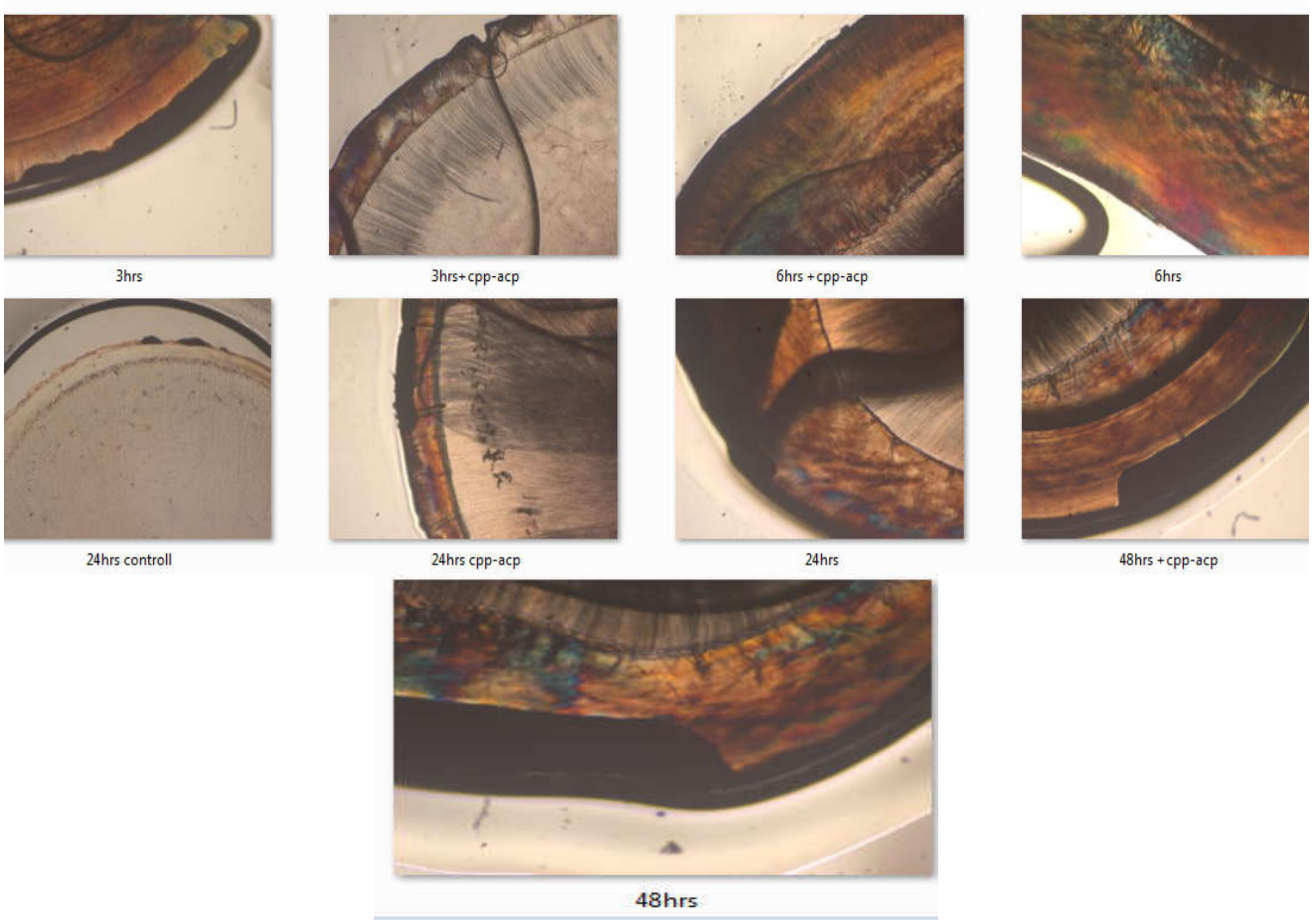

Figure 4. Comparison of D1 values of redbull drink and redbull+CPP-ACP at 48hrs with IMAGE PRORA software.

IMAGE PRORA software was used to measure the distance of demineralization of the microscopic images. Extent of D1 line of computer software will detect the degree of demineralization. Extent of demineralization was interpreted in pixels in the microscopic images. (Figure-3).

Statistical analysis

Independent t test was used to compare the P^H values among different groups. ANOVA was used to compare the degree of demineralization among the A, B, C. P value was set at 0.05 with 5% level of significance.

RESULTS

pH Values of the procedure –A and procedure-B shows an increase in values before and after the procedure (Table-1) whereas in procedure -C there is no change in pH values. Before the start of procedure, when we measure the pH values in both the groups, the pH values in procedure-A(redbull drink) and procedure-B(redbull+CPP-ACP) both shows values less than 7 which indicates that both were in acidic range, but when we compare between, procedure-A(redbull drink) and procedure-B(redbull+CPP-ACP), after the immersion time, pH values were increased in(redbull +CPP-ACP) and the solution is less acidic than redbull (A).

Polarized Microscopic Evaluation

Polarized microscopic evaluation of tooth samples were obtained at different immersion times. 3hrs, 6hrs, 12hrs, 24hrs, 48hrs.

The Extent of demineralization in the Polarized microscopic images measured with IMAGE PRORA software were indicated with D1 values which are represented with green line. D1 value at 48 hrs for acidic drink (Redbull) gives a value of 560.7 pixels, drink +CPP-ACP gives a D1 value of 435.247pixels. (Figure-4)

D1 values at 24 hrs for acidic drink (Redbull) gives a value of 327.98 pixels, Acid+CPP-ACP gives a D1 value of 191.393 pixels.

D1 values at 12 hrs for acidic drink (Redbull) gives a value of 228.93 pixels, Acid+CPP-ACP gives a D1 value of 151.91pixels.

D1 values at 6 hrs for acidic drink (Redbull) gives a value of 117.12 pixels, Acid+CPP-ACP gives a D1 value of 108.2 pixels.

D1 values at 3 hrs for acidic drink (Redbull) gives a value of 87.29 pixels, Acid+CPP-ACP gives a D1 value of 81.71pixels. These values indicate that there is a decrease in the loss of mineral content (D1value) of the drink+CPP-ACP when compared to D1 value of redbull drink.

(Figure 4) shows that Redbull drink shows a D1 value of 560.05 pixels. Redbull +CPP-ACP shows 435.24 pixels. This shows there is a decrease in demineralization of enamel surface of Redbull+CPP-ACP than Redbull drink. One way ANOVA used to measure Loss of mineral content of the Samples in between the groups and within the groups. ANOVA values were represented in (Table-2) The total mean of Loss of mineral content of samples were 152.6$\pm$171.7. In this, ANOVA of loss of mineral content shows F value of 4.997, P value of <0.05(=0.27), this shows that there is statistically significant difference in Loss of mineral content between the groups A,B measured. Sportsdrink+ CPP-ACP (B)(193$\pm$ 14) showed decreased amount of demineralization when compared to Sportsdrink (A)(264$\pm$19). This shows that CPP-ACP has greater efficacy in contrasting dental enamel erosion caused by acidic nature of sports drink.

DISCUSSION

pH values of tooth samples measured before the start of procedure were 2.54,2.53,2.54,2.55,2.54. After the completion of immersion time at 48hrs, 24hrs, 12hrs, 6hrs, 3hrs showed pH values to be 4.72,4.35,4.33,3.36,3.04. Significant difference between pH values were probably due to progressive loss of carbonates from the solution during immersion process. As sports drinks lowers oral pH, calcium ions were extracted from tooth surface into saliva to compensate for low oral pH environment. This process leaves a softened matrix for additional destruction thus results in enamel erosion. A study conducted by Lopes GC *et al* states that softdrinks, sports drinks, energy beverages can be destructive to children's and adolescent teeth since mineralization in immature permanent enamel is not complete, allowing an increased susceptibility from aggressive nature of beverages. (Lopes *et al*., 2007) In procedure-B, redbull +CPP-ACP had increase in pH values, indicates turning of sports drink to less acidic drink. This decrease in acidity of the sample was mainly due to release of calcium, phosphate ions from CPP-ACP complex into the drink solution. A study conducted by Barbour etal and Lussi A etal states that when calcium and phosphate are added , pH of the solution usually raises and thus decreasing acidity of solution. (Barbour *et al*., 2008; Lussi *et al*., 2000)

Prog Res CS Polarized light microscopic images of samples in group-A at 48hrs of procedure-A (Redbull) showed D1,distance of demineralised portion of enamel with a value of 560.057 pixels and this value has been decreased in procedure-B with a D1 value of 435.27 pixels which shows that demineralization process of enamel after the completion of immersion time had loss of minerals of enamel. Loss of minerals of enamel are greater in redbull drink than redbull+CPP-ACP. These findings are similar to the study conducted by Jayarajan *et al* that on comparing remineralisation value of CPP-ACP to that of demineralization value it is evident that a significant amount of remineralisation had occurred. (Jayarajan *et al*., 2011) In the present study, reduced loss of minerals in Procedure-B (redbull+CPP-ACP) is due to protective action of CPP-ACP. Calcium and phosphate ions from CPP-ACP complex are binded to the eroded areas of enamel caused by the acidic nature of drink.CPP forms nanoclusters with ACP thus providing a pool of calcium and phosphate. CPP will drive diffusion of ions Calcium and Phosphate into these demineralised areas. Increase in concentration of ions in eroded lesions will result in crystal growth of calcium and phosphate and helps in formation of hydroxyapatite thus results in remineralization. Study setting not done in a artificial salivary medium may be limitation of study as Saliva is one of the protective agent of tooth demineralization. Sports persons are commonly involved in strenuous exercises. Athletes will have better recovery and energy levels if they are constantly hydrated. Sports drinks will prevent dehydration and enhance the performance. Frequent and excessive consumption of acidic and sports beverages will result in dental erosion which leads to loss of tooth structure and sensitivity. CPP-ACP will facilitate remineralisation and helps to combat dental enamel erosion. A better protective action with CPP-ACP can be facilitated in immunocompromised patients too. Further invivo studies can be carried out to see CPP-ACP remineralisation effect in broader prospect.

Conclusion

Dehydration and tiredness are the conditions faced by sports persons in their activity. Sportsdrinks will refresh, prevent dehydration and enhance physical and mental performance of individuals. Dental erosion is the main problem faced due to decreased pH and loss of mineral content with these beverages. CPP-ACP has the ability to reduce demineralization caused by sports drinks and facilitates protective action of teeth with release of calcium and phosphate ions into eroded areas of tooth.

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