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RESEARCH ARTICLE

DEMINERALIZATION OF TOOTH ENAMEL SURFACE AT DIFFERENT IMMERSION TIMES OF SPORT DRINK AND BUFFERING EFFECT OF CASEIN DERIVATIVE AGAINST ENAMEL EROSION

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Abstract

Introduction: Sports drinks are becoming increasingly popular as well as being encouraged with regular exercise. These drinks are less than normal pH and found to be acidic.These acidic bevarages 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.

Objective: To estimate the pH values of Sport drink(Redbull) before and after immersion time. To estimate the loss of mineral content of the tooth exposed to sports drink with and without CPP-ACP.

Material & Methods: Extracted premolar teeth of human dentition free of dental caries and hypocalcification were selected. Teeth were sectioned to obtain 3 enamel sections from each tooth to obtain 15 sections. Specimens were immersed in Sports drink(Redbull) 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 analysed. After completion of immersion time, Tooth specimens were sectioned using Hard tissue Microtome; viewed for extent of demineralization under Polarized light microscope.

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. Sportsdrink+ CPP-ACP (B)(193+ 14) showed decreased amount of demineralisation when compared to Sportsdrink (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.[1] It may be caused by either extrinsic or intrinsic factors. One of the extrinsic causes of dental erosion is excessive consumption of acidic beverages.[2] 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.[3,4] . 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 a 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.[5]

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.[6] Cochrane et al states that recently, it was shown by immunolocalizationthat CPP were present inside a remineralized enamel subsurface lesion, indicating that they can navigate the size and charge impediments to enter the lesion.[7] The CPP-ACP nanocomplexes can be used to deliver high concentrations of bioavailable Calcium and Phosphate ions intraorally to inhibit demineralization and promote remineralization.[8] CPP-ACP releases Calcium and Phosphate ions into plaque fluid by equilibrium release, Competition release, pH difference and hydrolytic release.[6] 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.[9,10.] Sports drinks often result in erosion of toothsurfaces 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. Null hypothesis of the study is that CPP-ACP has no efficacy in contrasting dental enamel erosion of Sports drinks. The purpose of present study is to assess CPP-ACP efficacy in contrasting dental erosion caused by acidic nature of Sports drinks.

Material and Methods:-

Extracted premolar teeth which are free from hypocalcification, Caries any other initial enamel lesions are collected.

These teeth are collected and stored in 5% formalin solution in the sterile containers. These premolar teeth obtained were sectioned mesiodistally into three equal halves in order to obtain sections of tooth samples equally. 15 tooth samples were obtained in this study. The roots were cut and crowns were allowed for exposure to the acidic drinks.

A small window of 4x4mm dimension is prepared on the surface of sectioned teeth by coating all the surfaces with nail enamel varnish.

10ml of Sports drink (Redbull) was taken.0.25% of CPP-ACP is diluted in 99.75% of Redbull drink. The solution of Redbull+CPP-ACP is taken in a sterile conical flask and complete dilution of the drink and CPP-ACP is obtained with orbital shaker with 125rpm.(fig-1).

In the procedure, samples were divided into 5 groups, as group-1,group-2,group-3,group-4,group-5, based on immersion time of 48hrs,24hrs,12hrs,6hrs,3hrs. (table-1)Redbull drink categorized into groups form procedure-A, Redbull+CPP-ACP categorized into groups form procedure-B, Distilled water (control) categorized into groups forms the treatment-C.(fig -2)

The sectioned teeth were properly immersed according to the immersion time 48hrs, 24hrs, 12hrs, 6hrs, 3hrs. in all the procedure categories.The surface which is left with a window space is subjected to acidic drink and rest of the tooth is not subjected as it is coated with nail varnish.

Fig:-1:- Orbital shaker with conical flask.

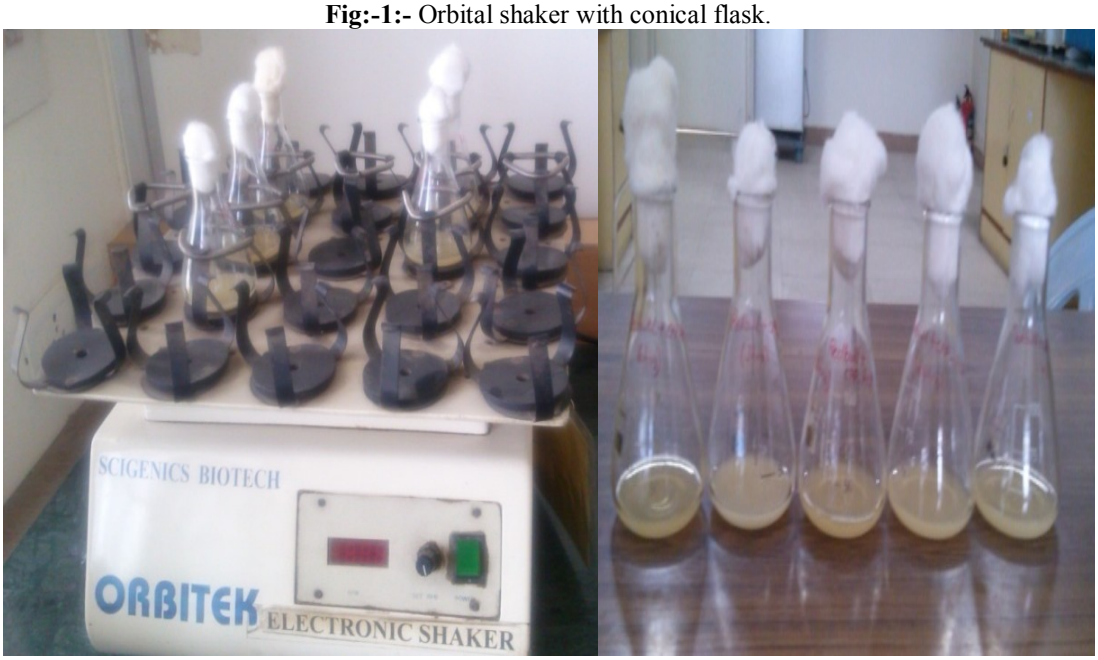


Fig-2:-Picture depicts Samples categorized into 3 procedures with 5 groups based on immersion time.

A=Redbull,B=Redbull+CPP-ACP,C=Control

Table1:- categorization of immersion time into 5 groups

	Immersion time	Treatment -A	Treatment-B	Treatment-C
Group-1	48 hrs	redbull	Redbull +cpp-acp	Distilled water
Group-2	24 hrs	redbull	Redbull +cpp-acp	Distilled water
Group-3	12 hrs	redbull	Redbull +cpp-acp	Distilled water
Group-4	6 hrs	redbull	Redbull +cpp-acp	Distilled water
Group-5	3 hrs	redbull	Redbull +cpp-acp	Distilled water

pH values of the Drink; Drink+CPP-ACP and distilled water is assessed with the help of digital pHmeter, before the start and end of the procedure(fig-3).The pHvalues of the samples are obtained and illustrated in (table:-2)

Fig 3:- Picture showing Digital pH meter.

Table 2:-PH values of the samples before and after start of the procedure.

PH VALUES BEFORE THE START OF PROCEDURE			AFTER THE START OF PROCEDURE:-					
CATEGORIES	IMMERSION TIME		procedure -A		procedure-B		procedure- C	
			BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
GROUP-1	48HRS	PH VALUES	2.54	4.72	2.63	4.8	6.99	6.99
GROUP-2	24HRS	PH VALUES	2.53	4.35	2.62	4.48	6.99	6.99
GROUP-3	12HRS	PH VALUES	2.54	4.33	2.61	4.37	6.99	6.99
GROUP-4	6HRS	PH VALUES	2.55	3.36	2.60	3.39	6.99	6.99
GROUP-5	3HRS	PH VALUES	2.54	3.04	2.64	3.02	6.99	6.99

pHvalues of the samples of Redbull(procedure-A),Redbull+CPP-ACP(procedure-B) and distilled water(procedure - C) are assessed and tabulated.

After the completion of immersion time , tooth samples were removed and the samples were subjected to hard tissue microtome sectioning. Tooth samples were mounted into acrylic blocks and thin sections were made with hard tissue microtome.(fig:-4). The thin sections of tooth obtained from microtome are mounted on glass slide and coverslip is placed. (fig-5)These microscopic slides are viewed under polarized light microscope(prog Res cs) and also under electron microscope. Prog Res cs microscope was connected to Computer and microscopic images are viewed.(fig-6).

Figure:4:- Hard tissue microtome and sectionnning the samples.

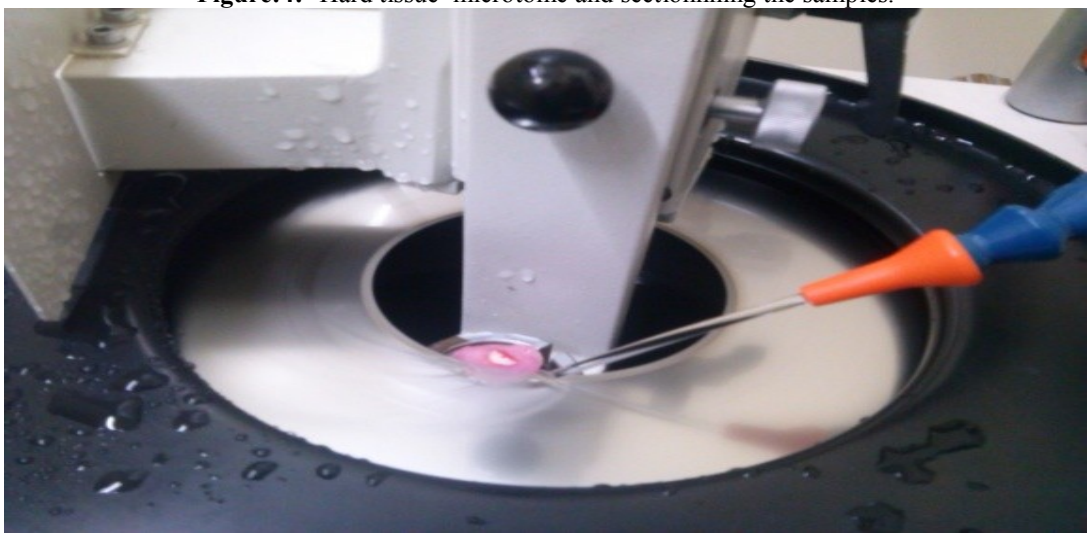


Figure-5:- Hard tissue microtome Sectioned tooth samples placed on the glass slide.

Figure-6:- Prog ResCS Polarised light microscope.

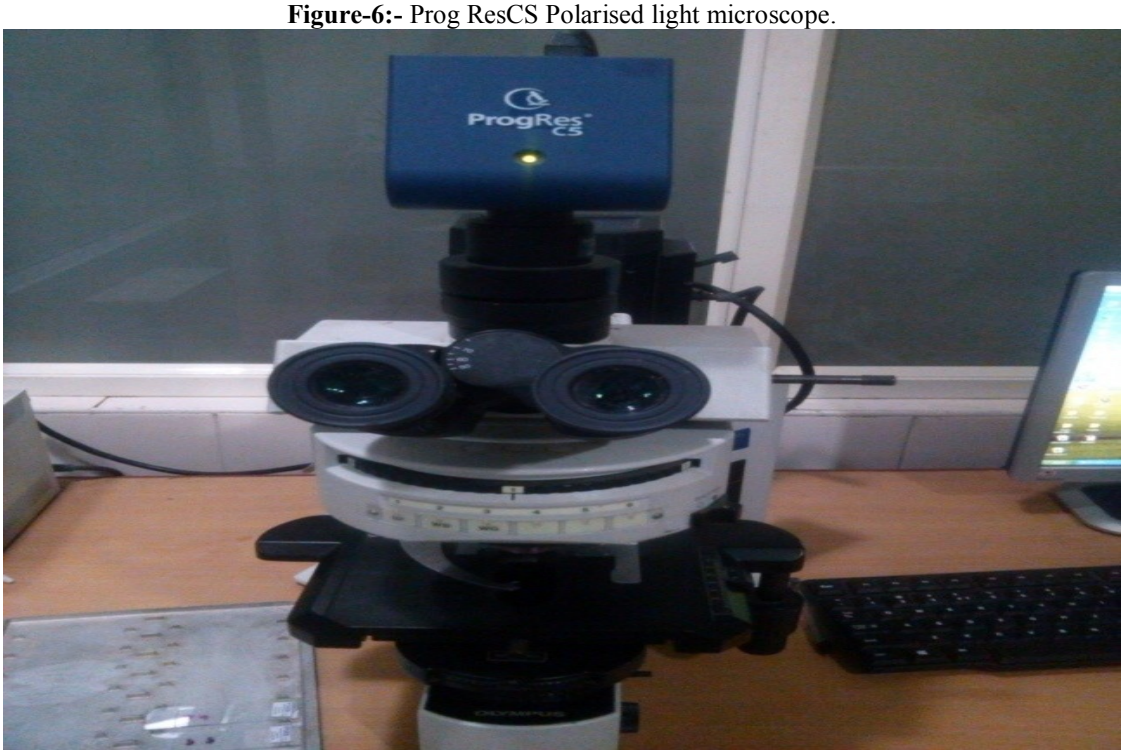
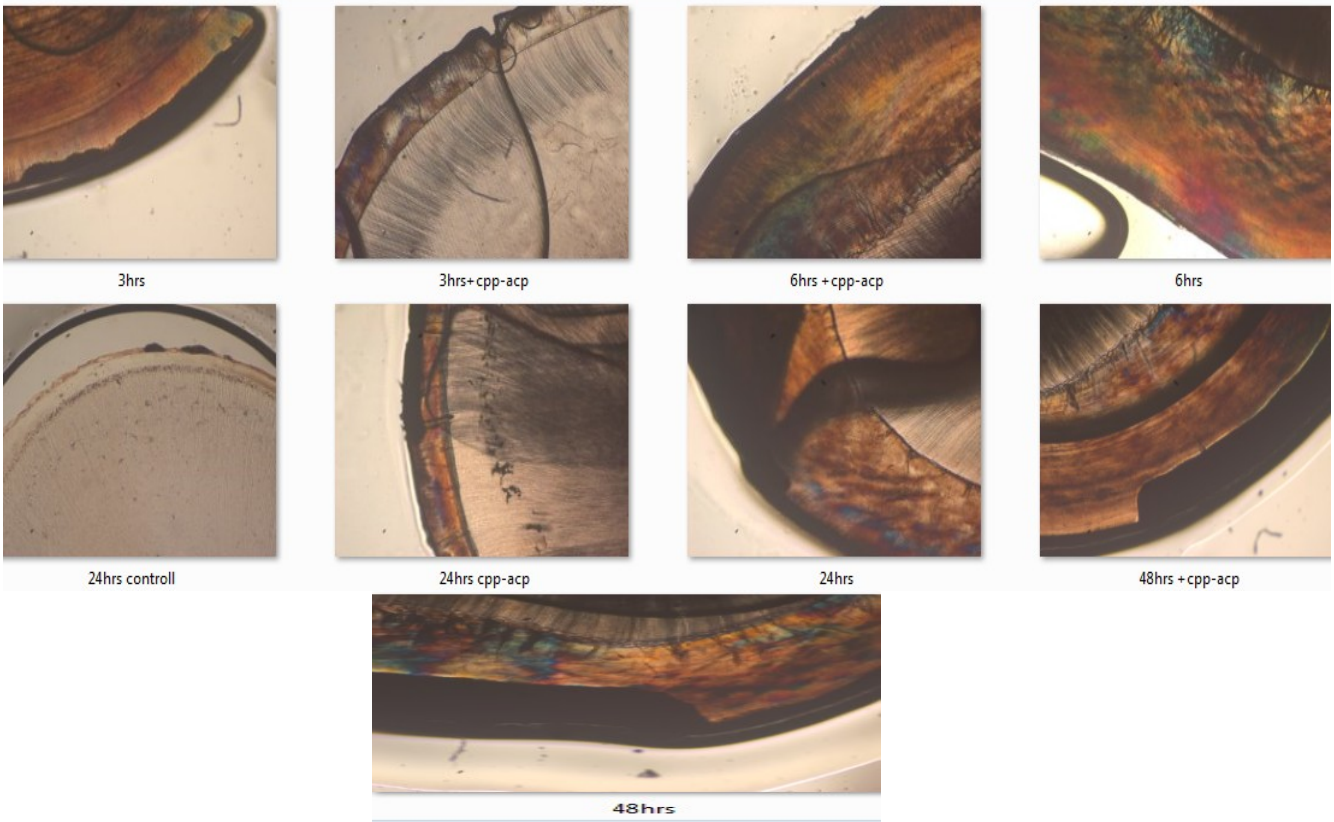


Figure-7:- Images viewed under polarized light microscope at each immersion time.



Comparison of groups based on immersion time and extent of demineralization were analysed (Fig-7) IMAGE PRORA software was used to measure the distance of demineralization of microscopic samples. Extent of demineralization in all microscopic images were interpreted in pixels. The demineralization of samples are viewed under polarized light microscope.

Statistical analysis:-
Independent t test was used to compare the pH values among different groups. ANOVA was used to compare the degree of demineralization among procedure groups.
P value is set at 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-3, 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).

Table-3:- 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

Polarized Microscopic Evaluation:-
Polarized microscopic evaluation of tooth samples were obtained at different immersion times. The figure-7 represents the polarized microscopic images at immersion times of 3hrs,6hrs,12hrs,24hrs,48hrs.

The Extent of demineralization in the Polarized microscopic images measured with IMAGE PRORA software indicated with D1 values.[Fig-8].D1 values of the tooth samples in the polarized images were represented with green line.

D1 values at 48 hrs for acidic drink (Redbull) gives a value of 560.7 pixels, Acid+CPP-ACP gives a D1 value of 435.247pixels.
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 values of redbull drink.

[Figure 9] shows that Redbull drink shows a D1 value of 560.05 pixels. Redbull+CPP-ACP shows 435.24 D1value. 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-3] The total mean of Loss of mineral content of samples were 152.6+171.7. In this table, loss of mineral content shows F value of 4.997, P value of <0.05(=0.27), this shows that there is a difference in Loss of mineral content between the groups A,B measured. Sportsdrink+ CPP-ACP (B)(193+ 14) showed decreased amount of demineralisation when compared to Sportsdrink (A)(264+19). This shows that CPP-ACP has greater efficacy in contrasting dental enamel erosion caused by acidic nature of sports drink

Fig:- 8:- Image Prora soft ware – D1 Values of extent of demineralization of the samples.

In the above image the green line indicates the extent of demineralization and D1 values are plotted with the IMAGE PRORA software.

Figure-9 :- Polarized Microscopic evaluation of D1 values of Redbull drink and Redbull+CPP-ACP at 48 hours

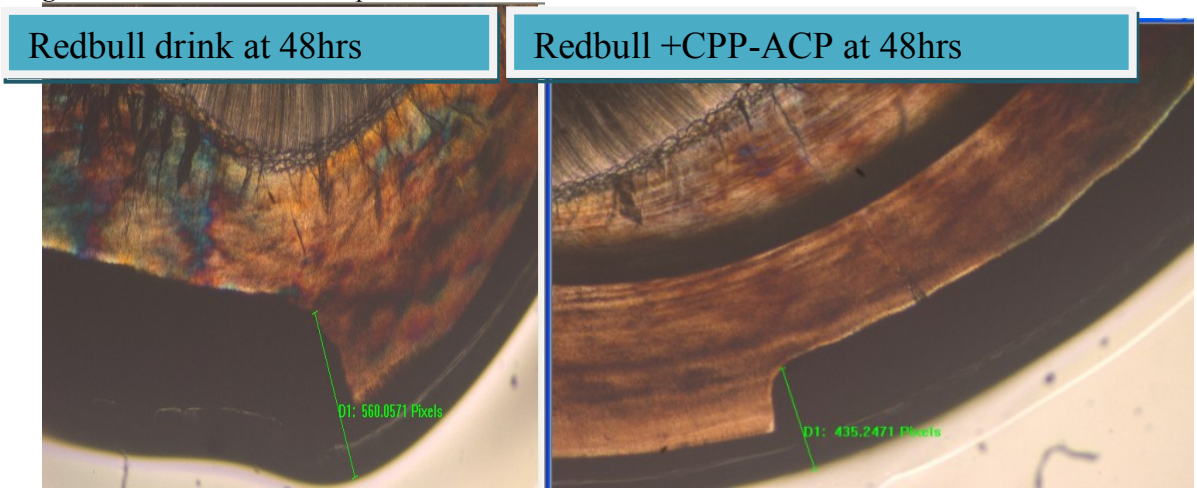


Table- 10:- One way ANOVA analysis of Loss of mineral content of the samples.

	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	5	193.6920	141.37600	63.22527	18.1505	369.2335	81.71	435.25

B								
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			

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 propably 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 etal 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.[11]

In procedure-B, redbull +CPP-ACP had increase in pH values. This increase in pH values indicates turning of sports drink to less acidic drink. Thus 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.[12,13]

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 in enamel are more in redbull drink than redbull+CPP-ACP. These findings are similar to the study conducted by Jayarajan etal ,that on comparing remineralisation value of CPP-ACP to that of demineralization value it is evident that a significant amount of remineralisation had occurred.[14] 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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Conflict of interest:-
None.

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