ORIGINAL ARTICLE

PROTECTIVE EFFECT OF TWO DIFFERENT REMINERALIZING AGENTS ON ARTIFICIALLY INDUCED DENTAL EROSION IN PRIMARY AND PERMANENT TEETH: AN IN-VITRO ANALYSIS

[1]SANDLEEN FEROZ

[2]FAISAL MOEEN

ABSTRACT

The main purpose of this in vitro study was to assess the effect of Chicken Egg Shell Powder (CESP) solution on demineralized enamel surfaces, as well as to compare its effect with those of commercially available Sensodyne Pronamel.

Twenty extracted human permanent pre-molars and 20 deciduous molars specimens were cleaned and sectioned longitudinally resulting in 80 samples.40 enamel sections obtained from permanent pre-molars and 40 from primary molars were assigned to the following four groups. Group A: demineralization by soft cola drink, Group B: demineralization by soft cola drink followed by direct application of Sensodyne Pronamel twice a day for a period of 7 days, Group C: demineralization by soft cola drink followed by 7 days' immersion in CESP, Group D: untreated.

The two treatments provided could increase the surface micro hardness values, Calcium and Phosphorous weight percentages in both the permanent and primary teeth. Both CESP (Group C) and Sensodyne Pronamel (Group B) exhibit significantly higher protective anti-erosive effects (p<0.05) but effects of Sensodyne Pronamel was significantly higher than CESP.

CESP significantly (p<0.05) increases the micro hardness as well as calcium and phosphorous level of all treated tooth samples. This study demonstrated that both CESP and Sensodyne Pronamel can protect the tooth surface by preventing its erosive enamel loss and enhancing remineralization.

Key Words: *Chicken Egg Shell Powder (CESP) solution, Enamel Demineralization, Energy Dispersive X – Ray Spectroscopy (EDX).*

INTRODUCTION

Dental Erosion also known as acid erosion is a process of chemical dissolution of tooth surfaces due to the acids produced by extrinsic or intrinsic sources.[1] Most commonly involved extrinsic agents are carbonated beverages, certain acidic food and frequent exposure to acidic environment. Intrinsic source to induce this erosion is mainly associated with gas1tric acid in oral cavity followed by vomiting, regurgitation or gastro esophageal reflux.[1] Although, teeth are exposed to continuous cycles of demineralization followed by remineralization, this delicate balance can easily be disturbed due to extensive use of low pH drinks like soft drinks, fruit juices, acidic beverages, wines and candies leading to acidic dissolution of the inorganic phase of tooth and subsequent loss of tooth substance.[2] This destructive process results in irreversible loss of tooth substrate thus increasing its susceptibility towards abrasion and may also cause erosive wear of dental hard tissues ultimately leading to tooth sensitivity and in severe cases pulpal exposure.[2] Many clinical complications associated with dental erosion are loss of esthetics, enamel fracture, shortening of the teeth leading to reduce vertical dimension, tooth sensitivity, difficulty in eating, pulpal inflammation and exposures mostly in children with large pulps.[4,5]

In recent years, loss of tooth substance due to dental erosion not only seen in adults but also in adolescent and children.[6,7,8,9] Studies showed wide range of erosion in both primary and permanent dentitions.[10,11] According to studies, primary dentition showed higher prevalence of dental erosion mainly due to reduce enamel thickness and its greater acid solubility as compare to permanent dentition.[8,11,12] Difference in the rate of progression of dental erosion between permanent and primary teeth is still a controversial issue in literature. According to some authors primary teeth enamel is more prone

[1] BDS, MPhil (Dental Materials), Lecturer, Department of Dental Materials, Fauji Foundation University College of Dentistry, Islamabad, Pakistan

[2] BDS, MSc (Singapore), Associate Professor, Department of Dental Materials, Islamic International Dental College, Riphah University, Islamabad, Pakistan **Corresponding Author:** Dr Sandleen Feroz, Department of Dental Materials, Fauji Foundation University College of Dentistry, DHA Phase 1, Islamabad, Pakistan Email: sandaleenferoz@yahoo.com

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to erosive wear as compared to permanent teeth[13,14], however others disagree.[15,16]

Recent management strategies in addressing early tooth decay appear to focus on the least invasive treatment options available. Loss of calcium and phosphate ions are mainly involved in the process of demineralization and their loss can be restored by using non-invasive calcium phosphate delivery system. Effectiveness of topical application of fluoride containing products as an anti-erosive agent has been proven successful both in vitro and in vivo. Thus, many studies have been carried out to determine the possible positive effects of topical application of fluoride containing products against acid erosion.[17,18,19]

In 1945 Gerould conducted a study using scanning electron microscopy (SEM) and observed the presence of calcium fluoride deposits on tooth surfaces after topical application of fluoride.[20] Later , several other studies also confirmed these findings.[21,22] Recently, many tooth creams have been introduced commercially which have claimed to prevent dental erosion.[23] Sensodyne Pronamel which is a derivative of Sensodyne tooth paste is specially designed for patients complaining about tooth sensitivity. It has greater concentrations of bioavailable fluoride and potassium nitrate (5%w/w).[24] These fluorides containing dentifrices also contains some effective element which are mostly added to increase the tooth surface resistance against acid erosion.

The outer crust of chicken egg is a non-edible product, mostly disposed of as a waste. It is a natural porous bio ceramic material which has largely been studied since 1969. The structure and chemical composition of egg shell has been studied in detail by using scanning electron microscopy and micro focus X-ray scattering techniques.[25]

Chicken egg shell is a well-organized structure, incorporating different soluble and in soluble proteins and minerals which is later used by the developing embryo. The deposited calcium is mainly used for the formation of embryo's skeleton.[25,26] According to the studies done to determine the chemical composition of egg shell it is mainly composed of in organic content which is mainly comprise of calcium carbonate (95%). The organic matrix which constitute about 3.5% composed of protein fibers lying, parallel to the egg surface thus providing structural support to the egg shell.[27]

The potential use of chicken egg shell has been investigated in various fields.[28] Chicken egg shell mainly composes of calcium carbonate crystals which constitute about 91% of the total mass. Due to its high calcium content chicken egg shell powder has a potential to use as human dietary calcium supplement especially for post-menopausal women and elderly population.[29] Several studies have been conducted to investigate the use of eggshell membrane to promote the healing of damaged tissues due to burns, ulceration or injuries.[30,31] similarly; study was conducted on female rats to increase the bone mineral density. CESP reduces osteo- resorption, enhances the cartilaginous growth and suitable in the treatment of osteoporosis to increase bone mineral density.[32]

An in vitro study was also conducted to evaluate the remineralization potential of chicken egg shell powder solution on demineralized enamel surfaces.[33] Enamel softening is one of the early manifestation of surface erosion and this reduced surface hardness can be assessed by using surface hardness tester.[19] In view of the above consideration, this in vitro analysis aimed to compare the anti-erosion effects of 0.1% sodium fluoride paste (Sensodyne Pronamel) and CESP on enamel lesions by the aid of micro hardness analysis and energy dispersive X-ray spectroscopy in both the permanent and primary teeth.

METHODOLOGY

a) Preparation of Chicken Egg Shell Powder (CESP) solution:

Calcination process given by World Property Intellectual organization (WO/2004/105912: Method of Producing egg shell powder) was followed for the preparation of chicken egg shell powder. Twelve chicken eggs used in this in vitro study were obtained from local hatchery (Islamabad Poultry Farms D-98, Satellite Town, Rawalpindi, Pakistan). After cleaning with distilled water, the egg shells were placed in hot water bath for 10 mins at 100°C to separate the membranes.

The egg shells were crushed with sterile mortar and pestle and then kept in a muffle furnace (Neycraft Model JFF 2000) at 1200°C. To prepare the solution One gram of CESP was dissolved in 20ml of 4% acetic acid. The clear fluid at the top of test tube was transferred to a beaker. The pH of the solution measured was about 11.4 by using a pH meter (Mettler Toledo)

b) Sample Preparation:

A total of 20 human deciduous molars used in the study were extracted from children 6-12 years old and the remaining 20 permanent caries free premolars were obtained from patients aged between 12-35 years. The parents gave their written consent that the teeth could be used for this study purpose. All the extracted teeth were cleaned from soft tissue debris by ultrasonic scaler and further disinfected by immersion in sodium hypo chloride solution (5%) for one hour.

The coronal portion of the collected teeth were separated from the radicular portion using a slow speed diamond saw (Laizhou weiyi Co. Ltd Model DTQ-5) with water irrigation so that 80 specimens were obtained from a total of 40 tooth samples as shown in Fig 1. All the enamel sections obtained were carefully inspected for any kind of enamel defects caries or white spot lesions. The cut surfaces were ground wet followed by polishing with alumina paste (Struers Als, Copenhagen, Denmark). The specimens were then positioned in pre-formed Teflon molds (10mmx 8mm x 2mm) and finally fixated with flow able composite resin.

c) Demineralizing Protocol:

A Coca Cola drink (Coca Cola Pakistan Ltd) was chosen as a demineralizing bath to induce dental erosion. The pH of the drink at 20°C was measured to be at 2.4 All primary and permanent tooth specimens were randomly assigned to the following four groups (n=20) as shown in Table 1. The enamel specimens in group D were not subjected to any treatment. However, Group A, B and C specimens were exposed to four consecutive cycles of demineralization by 2mins immersion in 6ml of soft drink carried out at 0,6,12 and 18 h intervals at room temperature.

The enamel sections in Group B were exposed to direct application of Sensodyne Pronamel (Glaxo Smith Kline, Weybridge, UK) twice a day for 3 mins with an aid of cotton tips and were later thoroughly rinsed by deionized water. The procedure was repeated twice daily for a period of 7 days. Followed by demineralization enamel sections in Group C were exposed to 100ml of chicken egg shell solution (CESP) for 7 consecutive days. Before and after exposure to different remineralizing or demineralizing solutions all specimens were stored in a 100ml deionizing water. All the procedures were done at a room temperature of 24°C.

d) Surface Hardness Assessment:

The surface micro hardness of all the samples were measured using Digital Display Vickers Micro Hardness Tester (Laizhou Huayin Testing Instruments Co., Ltd. China) with a diamond shaped indenter. A load of 25g was applied to the surface of the specimens for 5 seconds. Five indentations were made with a spacing of 100 microns for each sample. The diagonal length of the indentation made was then measured and Vickers values obtained were converted into micro hardness values.

e) Atomic Analysis by EDX:

Energy Dispersive X-Ray Spectroscopy (EDS) was used for elemental analysis of the surface deposits. All samples were stored in pre-labeled plastic containers containing deionized water at room temperature. All specimens were sputter coated with a thin layer of gold under vacuum, in a sputter coater (Q150T ES, Quorum, UK). Permanent and deciduous tooth specimens were then exposed to SEM scanning following mineral content evaluation (mainly calcium and phosphorous) by EDX (Quanta 200 FEG).

STATISTICAL ANALYSIS

a) Surface Micro Hardness Assessment and EDX Analysis

Data were analyzed and presented as mean and standard deviation values by using Statistical Package for Scientific Studies (SPSS 11.0 software).

ANOVA was conducted to compare any difference in the SMH, Ca weight percentage, P weight percentage, and mean molar ratios between the four different groups. This analysis was done separately for primary and permanent teeth. In cases where a significant difference was found ($p<0.05$), a post hoc analysis Tuckey test was conducted for inter group comparison. To compare any difference between SMH, Ca weight percentage%, P weight percentage % and mean molar ratios for all 4 groups between primary and permanent teeth, the 2 sample T test was conducted. Statistical results were considered significant at 0.05.

RESULTS

a) Statistical analysis of surface micro hardness of primary and permanent teeth:

One Way Anova test was carried out showing a statistically significant difference between all groups with a P – value of 0.001 (Table 2 & 4). Tuckey post hoc analysis was also conducted for inter group comparison of the surface micro hardness for primary teeth and permanent teeth. When comparing the SMH for primary teeth, all inter group comparisons were found to be significantly different except for the comparison between

TABLE: 1 ALL PERMENMENT AND PRIMARY TOOTH SPECIMENS WERE ASSIGNED TO FOLLOWING FOUR GROUPS

A: FOR SURFACE MICROHARDNESS AND EDX ANALYSIS (PERMANENTINCISORS + DECIDUOUS MOLARS)

	No. of Sample	Treatment Received	Application frequency	Application mode
Group A	10 Permanent 10 Deciduous	Demineralization only	4 consecutive cycles of 2 minutes' immersion in 6ml of soft drink	—
Group B	10 Permanent 10 Deciduous	Demineralization followed by application of Sensodyne Pronamel	Twice daily for 7 consecutive days	Direct application of paste on tooth surfaces
Group C	10 Permanent 10 Deciduous	Demineralization followed by 7 days' immersion in CESP	7 consecutive days	Immersion in solution
Group C	10 Permanent 10 Deciduous	No Treatment	—	—

TABLE 2: DESCRIPTIVE STATISTICS FOR SURFACE MICRO HARDNESS (SMH) OF PRIMARY TEETH

Group	Mean ± S.D.	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	182.70 ± 3.65	180.09	185.31
Group B (Sensodyne)	217.00 ± 11.27	208.93	225.07
Group C (CESP)	209.70 ± 7.21	204.54	214.86
Group D (Control)	298.30 ± 4.81	294.86	301.74

TABLE 3: INTERGROUP COMPARISON OF SURFACE MICRO HARDNESS (SMH) OF PRIMARY TEETH

Group	Comparison Group	Mean Difference	P Value
Control	Coca Cola	115.60	< 0.001
	Sensodyne	81.30	< 0.001
	CESP	88.60	< 0.001
Coca Cola	Sensodyne	-34.30	< 0.001
	CESP	-27.00	< 0.001
Sensodyne	CESP	7.30	1.36

TABLE 4: DESCRIPTIVE STATISTICS FOR SURFACE MICRO HARDNESS (SMH) OF PERMANENT TEETH

Group	Mean ± S.D.	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	196.90 ± 5.53	192.95	200.85
Group B (Sensodyne)	243.60 ± 9.88	236.53	250.67
Group C (CESP)	228.10 +±14.04	218.06	238.14
Group D (Control)	309.10 ± 8.52	303.01	315.19

Groups B and C where this difference was found to be statistically non-significant (Table 3 & 5). Statistical analysis revealed that all the tooth samples placed in demineralizing bath showed a significant loss of enamel

TABLE 5: INTERGROUP COMPARISON OF SURFACE MICROHARDNESS (SMH) OF PERMANENT TEETH IN DIFFERENT GROUPS

Group	Comparison Group	Mean Difference	P Value
Control	Coca Cola	112.20	< 0.001
	Sensodyne	65.50	< 0.001
	CESP	81.10	< 0.001
Coca Cola	Sensodyne	-46.70	< 0.001
	CESP	-31.20	< 0.001
Sensodyne	CESP	15.50	0.007

TABLE 6: COMPARISON OF SURFACE MICROHARDNESS (SMH) OF GROUP A BETWEEN PRIMARY AND PERMANENT TEETH

Group	Mean ± S.D.	P Value
Primary	182.70 ± 3.65	< 0.001
Permanent	196.90 ± 5.53	

TABLE 7: COMPARISON OF SURFACE MICROHARDNESS (SMH) OF GROUP B BETWEEN PRIMARY AND PERMANENT TEETH

Group	Mean ± S.D.	P Value
Primary	217.00 ± 11.27	< 0.001
Permanent	243.60 ± 9.88	

TABLE 8: COMPARISON OF SURFACE MICRO HARDNESS OF GROUP C (CESP) BETWEEN PRIMARY AND PERMANENT TEETH

Group	Mean ± S.D.	P Value
Primary	209.70 ± 7.21	0.003
Permanent	228.10 ± 14.04	

TABLE 9: COMPARISON OF SURFACE MICROHARDNESS OF GROUP D (CONTROL) BETWEEN PRIMARY AND PERMANENT TEETH

Group	Mean ± S.D.	P Value
Primary	298.30 ± 4.81	0.003
Permanent	309.10 ± 8.52	

TABLE 10: EDX ANALYSIS REGARDING THE ELEMENTAL CONCENTRATION OF CALCIUM (IN % BY WEIGHT) OF PRIMARY TEETH IN DIFFERENT GROUPS

Group	Mean ± S.D.	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	12.16 ± 0.95	11.48	12.84
Group B (Sensodyne)	35.13 ± 1.49	34.07	36.19
Group C (CESP)	32.60 ± 2.17	31.05	34.15
Group D (Control)	37.54 ± 2.55	35.71	39.37

TABLE 11: EDX ANALYSIS REGARDING THE ELEMENTAL CONCENTRATION OF PHOSPHOROUS (IN % BY WEIGHT) OF PRIMARY TEETH IN DIFFERENT GROUPS

Group	Mean ± S.D.	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	8.16 ± 0.66	7.69	8.63
Group B (Senso-dyne)	16.56 ± 1.11	15.76	17.36
Group C (CESP)	19.68 ± 1.62	18.52	20.84
Group D (Control)	16.12 ± 1.99	14.70	17.54

TABLE 12: CALCIUM/PHOSPHOROUS MEAN MOLAR RATIOS OF PRIMARY TEETH

Group	Mean ± S.D.	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	1.49 ± 0.13	1.40	1.59
Group B (Senso-dyne)	2.13 ± 0.75	1.97	2.30
Group C (CESP)	1.66 ± 0.14	1.56	1.76
Group D (Control)	2.36 ± 0.32	2.13	2.59

TABLE 13: POST HOC TUKEY TEST ANALYSIS FOR CALCIUM WEIGHT PERCENTAGE % OF PRIMARY TEETH

Group	Comparison Group	Mean Difference	P Value
Control	Coca Cola	25.38	< 0.001
	Sensodyne	2.41	0.035
	CESP	4.94	< 0.001
Coca Cola	Sensodyne	-22.97	< 0.001
	CESP	-20.44	< 0.001
Sensodyne	CESP	2.53	0.025

surface hardness as shown in Fig 3. Moreover, all the two treatments provided could significantly increase surface micro hardness values of Group B and C.

Comparing primary and permanent teeth SMH values for all 4 groups, the 2 sample T test was conducted. The SMH values of both teeth in group A showed a significant reduction. However, group A of primary teeth showed significantly lower values of SMH as compare to the permanent teeth in

TABLE 14: POST HOC TUKEY TEST ANALYSIS FOR PHOSPHOROUS WEIGHT PERCENTAGE% OF PRIMARY TEETH

Group	Comparison Group	Mean Difference	P Value
Control	Coca Cola	25.38	< 0.001
	Sensodyne	2.41	0.035
	CESP	4.94	< 0.001
Coca Cola	Sensodyne	-22.97	< 0.001
	CESP	-20.44	< 0.001
Sensodyne	CESP	2.53	0.025

TABLE 15: EDX ANALYSIS REGARDING THE ELEMENTAL CONCENTRATION OF CALCIUM (IN % BY WEIGHT) OF PERMANENT TEETH IN DIFFERENT GROUPS

Group	Comparison Group	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	9.16 ± 0.69	8.66	9.66
Group B (Senso-dyne)	15.36 ± 0.82	14.77	15.95
Group C (CESP)	17.48 ± 0.76	16.94	18.02
Group D (Control)	18.26 ± 0.62	17.82	18.70

TABLE 16: EDX ANALYSIS REGARDING THE ELEMENTAL CONCENTRATION OF PHOSPHOROUS (IN % BY WEIGHT) OF PERMANENT TEETH IN DIFFERENT GROUPS

Group	Comparison Group	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	9.16 ± 0.69	8.66	15.89
Group B (Senso-dyne)	15.36 ± 0.82	14.77	38.34
Group C (CESP)	17.48 ± 0.76	16.94	35.75

TABLE 17: CALCIUM / PHOSPHOROUS MEAN MOLAR RATIOS OF PERMANENT TEETH

Group	Comparison Group	95% Confidence Intervals	
		Lower Bound	Upper Bound
Group A (Coca Cola)	1.69 ± 0.19	1.55	1.82
Group B (Sensodyne)	2.45 ± 0.15	2.44	2.46
Group C (CESP)	2.02 ± 0.10	1.95	2.10
Group D (Control)	2.23 ± 0.24	2.12	2.33

TABLE 18: POST HOC TUKEY TEST ANALYSIS FOR CALCIUM WEIGHT PERCENTAGE % OF PERMANENT TEETH

Group	Comparison Group	Mean Difference	P Value
Control	Coca Cola	25.25	< 0.001
	Sensodyne	3.12	< 0.001
	CESP	5.30	< 0.001
Coca Cola	Sensodyne	-22.13	< 0.001
	CESP	-19.95	< 0.001
Sensodyne	CESP	2.18	0.002

TABLE 19: POST HOC TUKEY TEST ANALYSIS FOR PHOSPHOROUS WEIGHT PERCENTAGE% OF PERMANENT TEETH

Group	Comparison Group	Mean Difference	P Value
Control	Coca Cola	9.10	< 0.001
	Sensodyne	2.90	< 0.001
	CESP	0.78	0.096
Coca Cola	Sensodyne	-6.20	< 0.001
	CESP	-8.32	< 0.001
Sensodyne	CESP	-2.12	< 0.001

group A (Table 6). Similarly, both the group B and group C showed significant increase in surface micro hardness values after treatment with Sensodyne Pronamel and chicken egg shell powder (CESP) solution in both the permanent and primary teeth. However, mean surface micro hardness values of group B and group C in primary teeth was found to be less than the mean surface micro hardness values of permanent teeth in group B and group C as shown in (Table 7,8).

TABLE 20: EDX ANALYSIS REGARDING THE ELEMENTAL CONCENTRATION COMPARISON OF CALCIUM (IN % BY WEIGHT) BETWEEN PRIMARY AND PERMANENT IN DIFFERENT GROUPS

Group	Primary	Permanent	t.test	P. value
A (Coca Cola)	12.16± 0.95	15.36± 0.74	-8.40332	<0.001
B (Sensodyne)	35.13± 1.48	37.49± 1.19	-3.92979	0.001
C (CESP)	32.60± 2.16	35.31± 0.61	-3.81815	0.001
D (Control)	37.54± 2.55	40.61± 1.88	-3.06435	0.006

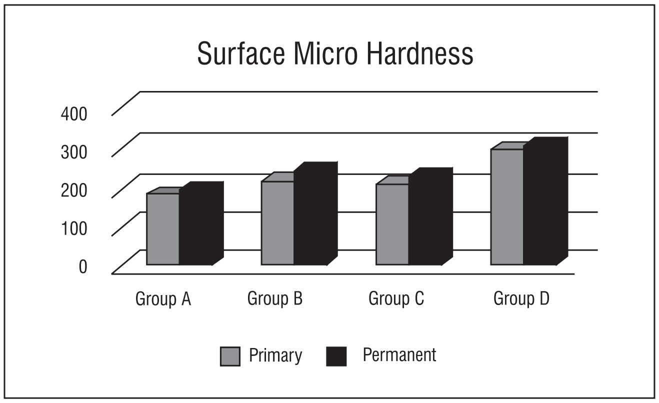

Fig 1: Comparison of the mean and standard deviation values of surface micro hardness (SMH) in primary and permanent teeth.

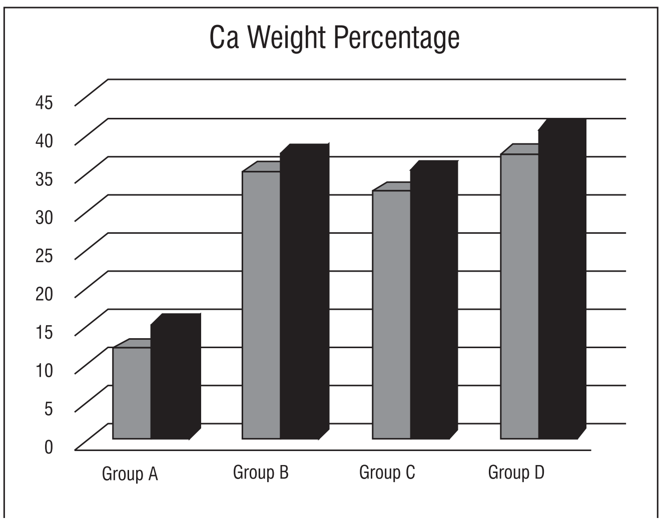

Fig 2: Calcium Weight Percentages of Primary and Permanent Teeth

b) Statistical evaluation of quantitative amounts of Calcium and Phosphorous (weight %) in primary teeth and permanent teeth

One Way Anova test was carried out to compare

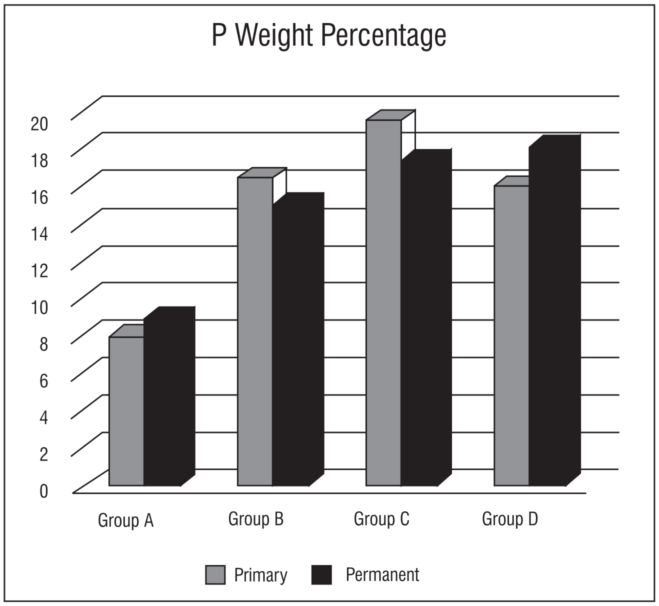

Fig 3: Phosphorous Weight Percentages of Primary and Permenent Teeth

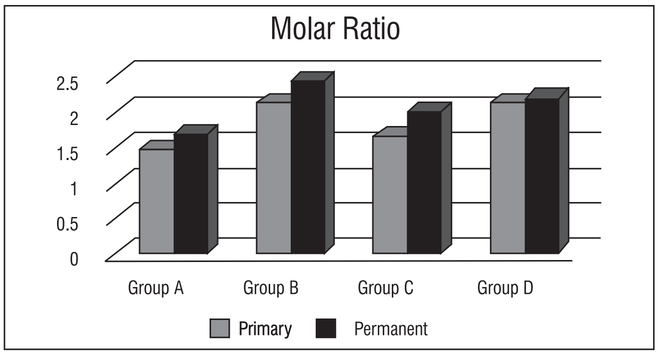

Fig 4: Calcium and Phosphorous Mean Molar Ratios of Primary and Permanent Teeth

any difference in the Calcium weight percentage %, Phosphorous weight percentage % and mean molar ratios between the four different groups suggested a statistically significant difference between all groups with a p-value of 0.01 (Table 10, 11, 12,15,16,17).

Tuckey post hoc analysis was used for inter group comparison for quantitative amounts of Calcium and Phosphorous of Group D to those of Group A as well as Group B and Group C (Table 13, 14, 18, 19). In primary teeth, statistical analysis regarding the elemental concentration of Phosphorous (in weight %) of Group D when compared with different groups showed a statistically significant difference except Group B (16.56±1.11) where this difference is statistically non-significant with a p-value of 0.001 (Table 14). Similarly, in permanent teeth statistical analysis regarding the elemental concentration of Phosphorous (in weight %) of Group D when compared with different groups showed a statistically significant difference except Group C (17.48±0.76) where this difference is statistically non-significant with a p-value of 0.001 (Table 19).

When comparing the permanent and primary teeth the difference in elemental concentration of Calcium and Phosphorous between all the groups were statistically significant at a p- value of 0.001 (Table 20, 21) Elemental concentration of Ca and P of Group B and C in primary teeth was found to be lower than the permanent teeth in Group B and C as shown in Fig 2, 3 & 4.

DISCUSSION

Dental Erosion also known as acid erosion is the irreversible loss of tooth substance due to the chemical dissolution by acidic drinks or beverages like soft drinks, wines, fruit juices, sports drinks.[3] If this condition remained unchecked, it may also proceed to underlying dentin. Several studies have been done to determine the underlying extrinsic and intrinsic causes of this acidic dissolution of tooth substance.[34,35,36,37] Demineralized tooth surfaces can be re hardens if treated early. Minor enamel defects are a physiological process and can be treated by certain dietary modifications and oral hygiene procedures. According to UK Child Dental Health Survey 8% prevalence of dental erosion was reported on palatal surfaces of primary teeth among the children of 2 years old and 52% in 5 years old. In permanent dentition the prevalence of this dental erosion was 8% on palatal tooth surface in 7 years old and up to 3% in 14 years old children.[38] Children from higher socioeconomic group of societies appears to have increased incidence of dental erosion.[39]

In this in vitro study, the anti-erosive effects of chicken egg shell powder solution were studied on both primary and permanent teeth. Moreover, the effect of CESP was compared to most commonly used tooth cream in dental practice nowadays containing sodium fluoride (0.1 % w/v fluoride ions) and potassium nitrate (5%).

The results of chemical analysis of CESP using X- Ray fluorescence spectroscopy revealed Calcium concentration of 98% and 0.4% of Phosphate.[40] This high concentration of bio available Ca ions plays a vital role in remineralization of eroded enamel surfaces. The process of calcination not only remove pathogens from chicken egg shell powder but also increases its alkinity. Addition of acetic acid (10%) to form chicken egg shell powder solution further ensured that powder is virtually free of pathogens.[41]

According to manufacturer's Sensodyne Pronamel containing sodium fluoride (0.1 % w/v fluoride ions) and potassium nitrate (5%) strengthens and re hardens enamel. It also protects enamel against the effects of acid erosion. Tooth hypersensitivity is the most common manifestation seen in patients with enamel loss. By using certain medications tooth hypersensitivity can be treated either by deactivation of intra dental nerve or by sealing the dentinal tubules to prevent the external stimuli (thermal, mechanical) to reach the nerves.[42,43,44,45,46,47] Studies done on animals have shown that potassium ions reduce the sensory activity by acting directly on nerves.48 Potassium ions in the form of potassium nitrate (5% concentration) are most commonly used worldwide as desensitizing agents.[49,50,51] Fluoride containing tooth pastes deliver calcium and phosphate ions to the demineralized tooth surfaces. Per several in vitro and in vivo studies done these products restore the defected enamel surface by forming amorphous calcium phosphate (ACP).[52,53,54] ACP then transforms into apatite mineral in the presence of moisture which resembles to that of natural enamel layer.[55]

All tooth surfaces used in the study were polished to minimize natural variation of surface enamel between different teeth.[17] However, natural tooth surfaces in the oral cavity show less erosive wear as compare to polished tooth surfaces.[18] In this study, one of the most commonly consumed beverage Coca Cola (Pak Ltd) was chosen as a demineralizing bath to induce surface erosion. A temperature of 20°C was kept constant as there appears to be relationship between rate of erosion and temperature variation of beverages.[19] Moreover, to minimize the buffering effect produced by ionic dissolution from tooth surfaces the carbonated drink was replenished for every 2 mins.[13]

In this in vitro analysis, the protective effect of CESP against acid erosion was compared with the commercially available product on both the permanent and primary teeth by using micro hardness tester and Energy Dispersive X Ray Spectroscopy.

An early manifestation of surface erosion is enamel softening which can be measured by using either Knoop or Vickers indenter. Both the indenter can be used for the measurement of micro hardness of non-metallic surfaces. In this present study Vickers micro hardness tester, has been used with the load of 25g applied for the period of 5 seconds on the specimen surfaces. Surface micro hardness (SMH) values of both the permanent and primary were measured for Group D receiving no treatment followed by Group A placed in a demineralizing solution. Additionally, the SMH reading for both the Group B and C were recorded to determine the changes occur after demineralization followed by the application of Sensodyne Pronamel and CESP.

The results of our study suggested a statistically significant difference in SMH values between the four different groups. This analysis was done separately for permanent and primary teeth. In case where as significant difference was found ($p<0.05$), a post hoc analysis Tuckey test was conducted for inter group comparison. When comparing the SMH for primary teeth, all intergroup comparison was found to be significantly different except for the comparison between Group B and C. Our results suggested that in primary dentition the protective effect of CESP and Sensodyne to increase the SMH values was statistically non-significant.

The results of our study showed that all the two treatments provided to Group B and C could diminish the loss of enamel hardness as compare to specimens in Group A receiving no treatment after demineralization in both the permanent and primary teeth. Our study results are in accordance with those of previous studies done to assess the protective effects of fluoride containing products on surface erosion. According to which fluoride can protect the enamel against acidic attack but cannot completely prevent the process of demineralization.[56]

In primary and permanent teeth, our results showed that all the tooth samples placed in demineralizing bath showed a significant loss of surface hardness. All the two treatments provided could significantly increase the surface micro hardness in Group B and C. When the surface micro hardness of Group D was compared to those of Group A, B and C both the Group B and C showed significant increase in SMH values as compare to Group A receiving no treatment after demineralization. Similarly, Sensodyne Pronamel (Group B) showed highest protective effects against erosive enamel loss as compare to CESP (Group C).

The protective effects of fluoride products applied topically is mainly due to the formation of a Calcium Fluoride (CaF_2) like layer on the tooth surfaces which act as a reservoir of fluoride ions. CaF_2 deposits released fluoride ions during an acidic attack on the tooth surfaces. These fluoride ions become incorporated into the mineral forming fluoroapatite resulting in a significant reduction to further dissolution of enamel surface. Additionally, CaF_2 act as a protective layer preventing the direct contact of acidic ions with the underlying enamel. CaF_2 layer formation depends mainly on the concentration of fluoride agents and its duration of application. High concentration fluoride agents and their prolong application results in the formation of more stable layer of CaF_2.[57]

Similarly, the protective effects of CESP lie in a fact that it can act as a reservoir of ions that prevents enamel demineralization and enhances the remineralization. Calcium and phosphate ions act as building blocks for remineralization process and found in saliva. In addition to erosive demineralization prevention, CESP also found to re mineralize the early enamel carious lesion.[33]

Comparing primary and permanent teeth SMH values of both teeth in group A showed a significant reduction. However, group A of primary teeth showed significantly lower values of SMH as compare to the permanent teeth in group A. Similarly, both the group B and group C showed significant increase in surface micro hardness values after treatment with Sensodyne pronamel and chicken egg shell powder solution (CESP) in both the permanent and primary teeth. However, mean surface micro hardness values of group B and group C in primary teeth was found to be less than the mean surface micro hardness values of permanent teeth in group B and group C as shown in Fig 3.

These results suggested that difference in surface micro hardness (SMH) between primary and permanent teeth in all groups were statistically significant (p value 0.001). This can be mainly because of two factors, firstly the structural difference between the permanent and primary teeth enamel. Enamel layer of deciduous teeth having higher porosity and lower degree of mineralization as compare to permanent teeth.[58] Secondly, the difference in the composition of enamel layer of these two dentitions which is mainly responsible for the variation observed to various protective treatments. Primary teeth enamel layer has higher content of carbon dioxide (CO2), carbonates and lower content of Calcium and Phosphorous. On micro crystal organization level, primary teeth enamel has less organized micro crystals arrangement and thus greater diffusion co-efficient when compared with the permanent teeth enamel. A prismatic layer of enamel on primary teeth erodes in an irregular manner and is more liable to erosive wear when compared to prismatic layer of permanent teeth enamel.[58]

The results of atomic analysis by EDX showed that quantitative amounts of Calcium weight %, phosphorous weight % and mean molar ratios is statistically greater for all the three groups except the demineralized group (Group A). In primary teeth results suggested that there is statistically significant difference in quantitative amounts of Ca (in weight %) in Group A, B and C when compared with Group D (Table 13). Similarly, statistical analysis regarding the elemental concentration of Phosphorous (in weight %) of Group D when compared with different groups showed a statistically significant difference except Group B (16.56±1.11) where this difference is statistically non-significant with a p-value of 0.001 (Table 14).

Similarly, in permanent dentition based on the results it could be suggested that there is statistically significant difference in quantitative amounts of Ca (in weight %) in Group A, B and C when compared with Group D (Table 18). Similarly, statistical analysis regarding the elemental concentration of Phosphorous (in weight %) of Group D when compared with different groups showed a statistically significant difference except Group C (17.48±0.76) where this difference was statistically non- significant with a p-value of 0.001 (Table 19).

Lastly, in this study effect of two different remineralizing agents on primary and permanent teeth were observed and compared having different developmental stages. An older tooth having in contact with the oral environment for much longer time as compare to newly erupted tooth showed more mineralization and more resistance against acidic attack.[59]

CONCLUSION

Based on our results, we conclude that both Sensodyne Pronamel and CESP showed a statistically significant increase in surface micro hardness values in both the permanent and primary teeth. Even though Sensodyne Pronamel showed more remineralization than CESP, the latter due to its natural source of Calcium and Phosphate ions and easy bioavailability can be the future in the prevention as well as treatment of surface erosion.

7. Conflicts of Interest

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CONTRIBUTIONS BY AUTHORS

1 **Sandleen Feroz:**	Idea, data compilation, material testing, wrote the article.
2 **Faisal Moeen:**	Research supervision & guidance.