Effect of Chicken Egg Shell Powder and Gallic Acid as Remineralising Agent on Early Enamel Carious Lesion- An In Vitro Study

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Abstract

Background: The aim of the study was to compare the effect of chicken eggshell powder and gallic acid as remineralising agent on early enamel caries lesion.

Material and Methods: Ten extracted deciduous molars was cleaned using distilled water. The teeth were decoronated at CEJ. Each decoronated tooth then sectioned longitudinally in a mesio-distal direction first and then in a bucco-lingual direction with diamond disc so that four samples obtained from a single tooth. Then from these samples, four enamel blocks of dimensions (4mm long, 4mm width and 2mm thick) made from each sample and embedded in acrylic blocks. These 40 samples are divided into four groups, each having 10 samples (GROUP A,B,C,D). Samples were demineralised followed by application of chicken egg shell solution and gallic acid. Chicken egg shell has the highest content of Calcium present as compared to Gallic acid.The ratio of Calcium/Phosphorous was found to be increased in the following sequence. Chicken egg shell>Gallic acid>Control>Demineralised group.

Conclusion: It can be concluded from the results shows that Calcium has a vital role in remineralisation.

Keywords*: Caries, Demineralisation, Remineralisation, Calcium*

Introduction

Dental caries is defined as microbial disease of the calcified tissue of the teeth characterized by the demineralization of the inorganic portion and distruction of the organic portion of the tooth.[1]Occurrence of white spot lesions is caused by colonization of pathogenic bacteria and their bonding in form of a biofilm to the enamel surface. During their metabolic activities the bacteria create surplus of organic acids and lower pH, while the biofilm prevents the effect of buffering mechanisms from saliva. Lowered pH dissolves the surface layers of calcium and phosphorous.[2]

Early diagnostic of a white spot lesion is extremely important because if the treatment is initiated on time, remineralization is encouraged and the cracking of the surface layer and the development of cavities are avoided.[3] At physiological conditions, the oral fluids (saliva,biofilm fluid) have calcium (Ca) and phosphate (Pi) in supersaturated concentrations with respect to the mineral composition of enamel and as a result, these ions are continually deposited on the enamel surface or are re-deposited in enamel areas where they were lost. In this study we have used gallic acid and chicken egg shell powder as remineralising agent. Gallic acid (3,4,5-trihydroxybenzoic acid, GA), a common naturally occurring polyphenol, is widely available in various plants and foods and it possesses a variety of biological properties, such as antioxidant, anti-mutagenic and anti-carcinogenic effects.[4]GA is stable at high pH and how

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potential chemical changes of GA will impact on its anti-caries effect.[5]Similarly, the use of CESP in various fields has been studied.

Chicken egg is a known source of Ca containing about 39%(w/w) of elemental Ca. Chicken Egg Shell contains about 95% of calcium carbonate which on conversion to basic calcium oxide due to calcination is responsible for an increase in alkalinity.[6] Eggshells contain the perfect amount of the ideal substances for healing cavities massive amount of calcium, protein and 27 other minerals which resembles the composition of our teeth. CESP reduces pain and osteoresorption and also increases bone mineral density .[7] Demineralized tooth surfaces have been studied widely by using technique sensitive instruments such as scanning electron microscope. Therefore this study was conducted with the objective to compare the effect of Chicken egg shell powder and Gallic acid as remineralising agent on early enamel carious lesion on the extracted deciduous molar tooth.

Material and Method

The present invitro study was conducted in the Department of Pedodontics and Preventive Dentistry, KD Dental College & Hospital, Mathura from November 2016 to March 2018. Ten extracted deciduous molars used for the study and cleaned with distilled water. The teeth were decoronated at CEJ. Each decoronated tooth then sectioned longitudinally in a mesio-distal direction first and then in a bucco-lingual direction with diamond disc so that four samples obtained from a single tooth. Then from these samples, four enamel blocks of dimensions (4mm long, 4mm width and 2mm thick) made from each sample and embedded in acrylic blocks and like this 40 samples are prepared embedded in acrylic blocks. These 40 samples are divided into four groups, each having 10 samples (GROUP A,B,C,D; Figure 1).

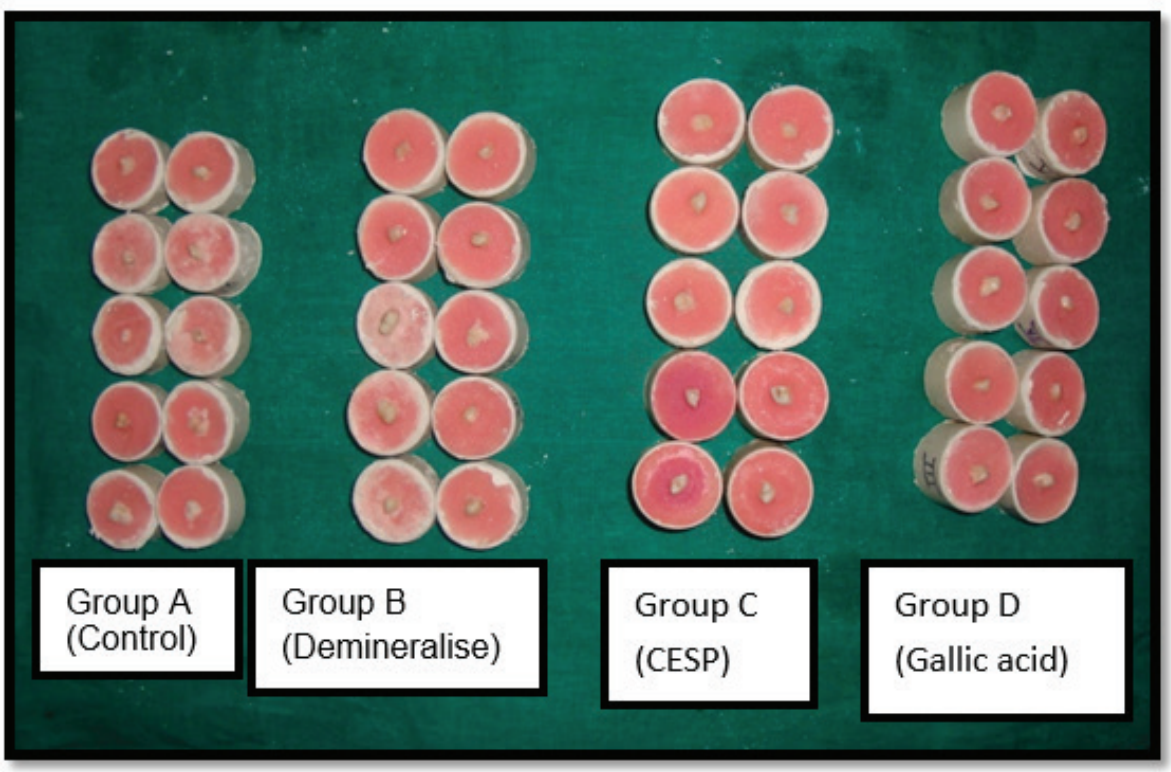

Figure 1: Cross sectioned tooth is embedded in acrylic blocks

Production of Egg Shell Powder: Twenty fresh egg shell was taken and cleaned it with distilled water and dried it. The eggshells then kept in hot water bath at 100 degree Celsius for 10 min followed by removing the inner membrane. The egg shells were then crushed using the sterile motar and pestle. The crushed particles then heated at 1200 degree Celsius using muffle furnace (G L Bajaj Institute and Technology Mathura) and powdered to small particles (Figure 2). Egg shell powder weight to one gram. This one gram of CESP was dissolved in 20 ml of 4% acetic acid in a test tube the clear fluid which is collected at the top was then transferred to a beaker and the ph of the solution was tested using a ph meter which was 11.7.

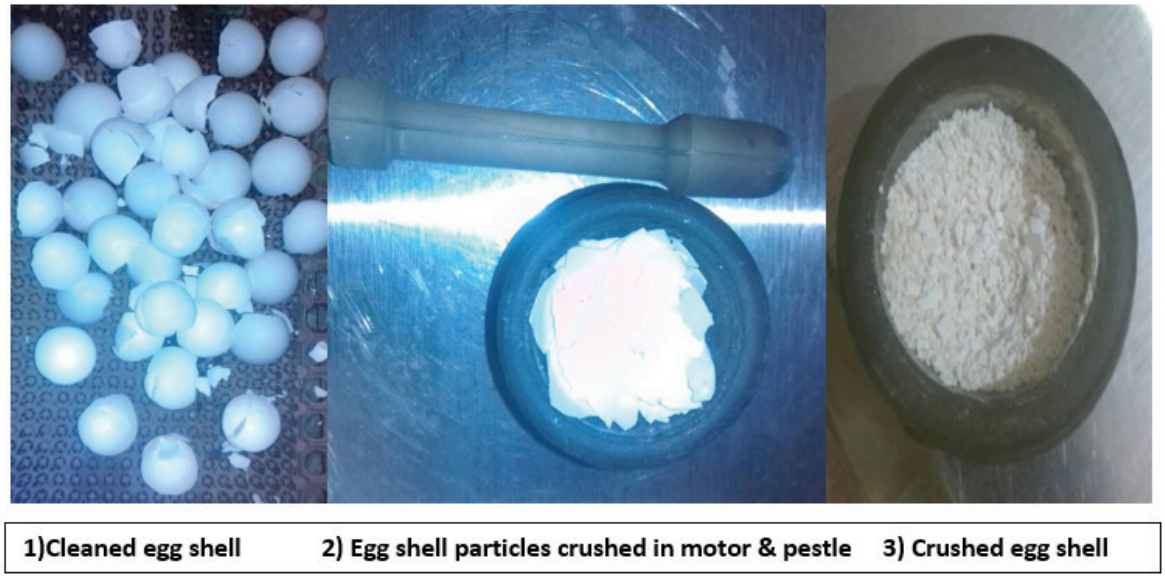

Figure 2: Crushing of egg shells

DEMINERALISING SOLUTION:

Demineralising solution was made by including (CaCl2, NaH2PO4, Lactic acid and Fluoride).

1) Fluoride -.002 gm of sodium fluoride in 100ml d/w.

2) CaCl2 (10%) - 10 gm 0f calcium chloride in 100 ml d/w.

2.2 ml in 1 litre d/w-2.2Mm

3) NaH2PO4 - Sodium Hydrogen Phosphate(10%)

0.3 ml in 100 ml of d/w-2.2Mm

4) Lactic acid - (0.05 mole)

0.45ml lactic acid in 100 ml d/w.

DEMINERALISING PROTOCOL:

Carious lesions representing preliminary stage of subsurface enamel lesion were created by placing the tooth samples in 20ml of demineralization bath for 72 h (CaCl2 = 2.2 Mm NaH2PO4 = 2.2 Mm Lactic acid = 0.05 M, Fluoride = 0.2 ppm, solution is adjusted with 50% NaOH to a pH of 4.5). The specimens kept in the demineralization solution (CaCl2, NaH2PO4, Lactic acid and Fluoride) for 72 h at 37° C created a subsurface demineralization (Figure 3).

Study groups: (n=40 enamel blocks)

Group A (n=10)- untreated group

Group B (n=10) - subsurface demineralization only.

Group C(n=10)–subsurface demineralization followed by suspending the tooth samples in Chicken Eggshell Solution for 21 hours for seven consecutive

days for remineralization.

Group D(n=10) -subsurface demineralization followed by application Gallic acid for 21 hours seven consecutive days for remineralization.

GROUP B GROUP CGROUP D

Figure 3: SUB SURFACE DEMINERALISATION OF THE SAMPLES FOR 72 Hrs

Chemical Analysis: Total Metals (Calcium, Phosphorus, Potassium, Magnesium)

1. Collect and weigh dentin chips in Microwave Digestion vessels.

2. Add 10 ml HNO3 (65 %) & 15 ml DI water and close the vessels.

3. Digest sample in microwave digestion at 220 PSI and 2000C for 30 min. After completion of digestion cool and mark to the volume.

4. Plot ICP Calibrate curve for the desired elements with CRM Standards and run dentin digest sample. Collect the results and quantify the elemental concentration percentage by weigh.

5. Metals were determined by inductively coupled plasma spectroscopy (ICP).

Major Equipment's / Apparatus Used:

1. Microwave Digestion (Make- CEM Mars 5)

2. Inductively coupled plasma spectroscopy (Make- Varian MPX Vista CCD Simultaneous ICP-OES).

Statistical analysis: Data were computerized and analysed using SPSS 24.0 software. The results were analysed using one way ANOVA and Tukey – Kramer multiple comparison test.

Result and Discussion

The mean value for Phosphorous content in Group A is 15.89, Group B is 8.55, Group C is 16.07, Group D is 32.6. The Phosphorous content in Group A (Control), B (Demineralised), C(CESP), D (Gallic acid) have significant (p) value 0.176 which is more than 0.05 therefore it is statistically not significant (Table 1)**.**

Table 1: Comparison of Phosphate ratio among all the Groups

Group	Number	Mean	SD	Anova test	p value
Group A (Control)	10	15.89	.21	1.739	.176
Group B (Demineralisation)	10	8.55	.14		
Group C (CESP)	10	16.07	.21		
Group D (Gallic acid)	10	32.68	48.95		
Total	40	18.29	25.16		

Table 2 shows increase in Calcium level is more in Group C(CESP)>D (Gallic acid)>A (Control)>B (Demineralised) and the significant (p) value is < .05 which is statistically significant.

Table 2: Comparison of Calcium ratio among all the groups

Group	Number	Mean	SD	Anova Test	p value
Group A (Control)	10	36.820	.2658	2.157E4	<.01*
Group B (Demineralisation)	10	17.050	.2014		
Group C (CESP)	10	37.760	.1955		
Group D (gallic acid)	10	35.000	.1700		
Total	40	31.658	8.6024		

*: statistically significant

Table 3 shows the intragroup comparison of mean values of calcium content by applying TukeyHsd Test. The statistical significant difference is 0.00 which is less than p<.05. The test is applicable for the comparition of Calcium content when intragroup comparison is done among all Groups. Calcium content is greater in Chicken egg shell as compared to Gallic acid , Control and Demineralised Group. Therefore CESP has more effect as a remineralising agent then Gallic acid.

Table 3: Multiple comparison test for Calcium (using Post Hoc test)

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ca(Calcium)	A (Control)	B	19.77*	.000*	19.516	20.024
		C	-.94*	.000*	-1.194	-.686
		D	1.82*	.000*	1.566	2.074
	B (Demineralisation)	A	-19.77*	.000*	-20.024	-19.516
		C	-20.71*	.000*	-20.964	-20.456
		D	-17.95*	.000	-18.204	-17.696
	C (CESP)	A	.94*	.000*	.686	1.194
		B	20.71*	.000*	20.456	20.964
		D	2.76*	.000*	2.506	3.014
	D (Gallic acid)	A	-1.82*	.000*	-2.074	-1.566
		B	17.95*	.000*	17.696	18.204
		C	-2.76*	.000*	-3.014	-2.506
*: statistically significant						

Table 4 shows the intragroup comparison of mean values of phosphorous content by applying TukeyHsd test. The statistically significant difference is more than 0.05.

Table 4- Multiple comparison test for Phosphorus (using Post Hoc test)

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
P (Phosphorous)	A (Control)	B	7.34	.908	-22.14	36.82
		C	-.18	1.000	-29.66	29.30
		D	-16.79	.429	-46.27	12.69
	B (Demineralisation)	A	-7.34	.908	-36.82	22.14
		C	-7.52	.901	-37.01	21.96
		D	-24.13	.141	-53.61	5.35
	C (CESP)	A	.18	1.000	-29.30	29.66
		B	7.52	.901	-21.96	37.01
		D	-16.61	.438	-46.09	12.87
	D (Gallic acid)	A	16.79	.429	-12.69	46.27
		B	24.13	.141	-5.35	53.61
		C	16.61	.438	-12.87	46.09

In this study we have compared the calcium and phosphorous percentage by mass in the tooth ingredient with chicken egg shell[7] and gallic acid[8] by ICP (Inductively Coupled Plasma Spectroscopy). According to our study CESP has 37.7 % by mass and the result is statistically significant with p value of <0.01 by post hoc method. The same method ICP was used by BrunaGressler(2015) and the Ca concentration reached 37% egg shell powder at an average of all analyzed samples (365,7mg g).[9] This content was similar to concentrations found by Schaafsma et al[10] (2000), on average 390 mg g^{-1}eggshell powder devoid of membranes, as analyzed in this study.[10]According to BenjoyMony (2015)[6]Ca % by mass was 37.67 which is approximately similar to our result. In this study atomic analysis by EDX(energy dispersive X-ray spectrometry) was done which shows highest Ca concentration of 98%. According to SandleenFeroz (2017)[11]the protective effects of chicken egg shell powder solution on enamel was compared with samples treated with demineralizing solution and the results showed a statistically significant difference (0.158±0.012) with a p value of 0.00 indicating the effectiveness of remineralizing effect of CESP on enamel surfaces. The statistical results were similar with our results. X- Ray fluorescence spectroscopic analysis of CESP revealed that it contains about 98% Calcium, 0.46% of phosphate, 0.53% of Magnesium, 0.18% of Strontium, 0.03% of Potassium .This high concentration of bio available Ca plays a vital role in enamel remineralization when CESP applied topically. Availability of calcium and phosphate ions is essential for remineralization to occur and increased pH of the solution along with rich bioavailability of phosphate and calcium ions is mainly involved for the process of remineralization.[6]

We proposed a hypothesis that GA might regulate the demineralization/remineralization by influence the growth of HAP crystals. Chemical composition analysis was done by X-ray spectrometer and SEM (scanning electron microscope) which allowed the relative amounts of calcium (Ca) and phosphorus (P) to be determined. The Ca/P ratios were also calculated. The results showed that in all the groups, the main elements of the precipitate were calcium and phosphorus, and the Ca/P ratios were close to 1.67 which is consistent with the Ca/P ratio in HAP**(Bei tang)[12].** The results we had observed Ca/P ratio by ICP method was approx 2.01 which was not similar to this study.It proposed that G. chinensis has anti-bacterial properties to restrain the growth and acid production of certain cariogenic bacteria, including streptococcus and lactobacillus. In particular, it reportedly has the ability to inhibit enamel demineralization in vitro. In our study also calcium and phosphorous levels are more thendemineralised group but less then chicken egg shell which shows that it helps in inhibiting demineralization and act as a good antioxidant.

Within the limitations of this study, it is concluded that the chicken egg shell solution along with the rich bioavailable calcium content has the potential to favouredremineralisation. Even though CESP showed more remineralization than gallic acid, the latter due to its easy bioavailability and natural source of calcium and phosphate can be the future in remineralisingenamel carious lesions. Further, clinical studies regarding suitable vehicle for CESP is needed as this might increase the remineralization potential of CESP comparable to the commercially available agents.

Conclusion

It can be concluded from the results that calcium has a vital role in remineralisation. Chicken egg shell has the highest content of Calcium present as compared to Gallic acid, Demineralised and Control group when the samples were tested by ICP (Inductively Couples Plasma Spectroscopy) method.For remineralization to occur, bioavailable calcium and phosphates are essential. Therefore, the rich bioavailability of calcium along with the high concentration of phosphates present in CESP solution coupled with its increased pH may be responsible for remineralization. Further, clinical studies regarding suitable vehicle for CESP is needed as this might increase the remineralization potential of CESP comparable to the commercially available agents.

Conflict of Interest: Nil

References

1) Houte VJ**.** Role of micro organisms in caries etiology. J Dent Res 1994;73(3):672-81.

2) Vojinović V, Čupić S, Mirjanić Đ, Sukara S, Dolić O, Obradović M. Remineralization of early caries lesions with glass ionomer cements. Contemp Mater. 2010;1:175-8.

3) Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions?.Braz Oral Res 2009;23:23-30.

4) Gao SS, Qian LM, Huang SB, Yu HY. Effect of gallic acid on the wear behavior of early carious enamel. Biomed. Mater. 2009;4:034101.

5) Zhang J. Changes in composition and enamel demineralization inhibition activities of gallic acid at different pH values. Actaodontologicascandinavica. 2015; 73: 595–601.

6) Mony B, Ebenezar AR, Ghani MF, Narayanan A. Effect of chicken egg shell powder solution on early enamel carious lesions: an invitro preliminary study. Journal of clinical and diagnostic research: JCDR. 2015;9(3):ZC30.

7) Harrington R. Did you know you can heal cavities with chicken egg shell.http./complete health and happiness.com. Last accessed onJune 7, 2015.

8) Nayeem N, Asdaq SM, Salem H, AHEl-Alfqy S. Gallic acid: a promising lead molecule for drug development. J App Pharm 2016;8(2):1-4.

9) Milbradt BG, Muller AL, da Silva JS, et al. Eggshell as calcium source for humans: mineral composition and microbiological analysis/Casca de ovocomofonte de calcioparahumanos: composicao mineral e analisemicrobiologica. Ciencia Rural. 2015;45(3):560-7.

10) Schaafsma A, Pakan I, Hofstede GJ, Muskiet FA, Van Der Veer E, De Vries PJ. Mineral, amino acid, and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. Poultry science. 2000;79(12):1833-8.

11) Feroz S, Moeen F, Haq SN. Protective effect of chicken egg shell powder solution (CESP) on artificially induced dental erosion: an in vitro atomic force microscope study. International Journal of Dental Sciences and Research. 2017;5(3):49-55.

12) Tang B. Control of hydroxyapatite crystal growth by gallic acid. Dental Materials Journal 2015; 34(1): 108–113.