International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Antibacterial and remineralization efficacy of Calcium Glycerophosphate mouth wash on eroded enamel

[1]Richa Goel, [2]Prabu Mahin Syed Ismail, [3]T Uma Arasu,

[4]Vaibhav T Chougule, [5]Amol Jain, [6]Kaushik Shetty*

[1]Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India
[2]College of Dentistry in Arrasss, Qassim University, Kingdom of Saudi Arabia
[3] Assistant Professor, Department of Microbiology, Swamy Vivekanandha Medical College Hospital and Research Institute, Elayampalayam, Namakkal District, Tamilnadu, India.
[4]Bharati Vidyapeeth Deemed to be University Dental College and Hospital (BVDUDCH), Sangli, Maharashtra, India
[5]Sardar Patel Post Graduate institute of Dental and Medical Sciences, Lucknow, Utter Pradesh, India
[6]AB Shetty Memorial Institute of Dental Sciences, NITTE (Deemed to be University), Derlakatte, Karnataka, India

Abstract

Excessive soft drinks consumption can cause erosion of tooth enamel. This in vitro study set out to assess calcium glycerophosphate's (CaGP) antibacterial and remineralization effects on permanent enamel that had been damaged by a soft drink. To develop erosive lesions, 48 sound permanent premolars were encased in self-curing acrylic resin and submerged in Coca-Cola. It was divided into 4 groups with 12 samples in each. Group A received artificial saliva; Group B received amine fluoride; Group C received sodium monofluorophosphate; and Group D received sodium monofluorophosphate + CaGP. For eight days, the specimens in the designated groups were subjected to pH cycling. Surface microhardness (SMH) values before erosion, after erosion, and after remineralization were ascertained. Each mouthwash's antibiofilm with a zone of inhibition against Streptococcus mutans was tested for 24 hours, and the impact was measured by counting the number of colony-forming units. ANOVA, or one-way analysis of variance, was used to analyse the data.The mean SMH values were significantly lower in all groups after being eroded (Demieralised) by the cola soft drink. Group A had the lowest %SMHR upon remineralization. Groups B, C, and D exhibited significantly greater %SMHR compared to Group A ($P < 0.001$). But among Groups B, C, and D, there were no noteworthy variations ($P > 0.05$). The effectiveness of fluoride mouth rinse in remineralizing eroded permanent enamel was comparable when it was used with or without CaGP.

Keywords: Amine fluoride, Calcium glycerophosphate, enamel, erosion, remineralization.

Full length article *Corresponding Author, Kaushik Shetty e-mail: drkaushik@nitte.edu.in

1. Introduction

The population's changing dietary habits and unique lifestyles have led to a significant increase in the overconsumption of soft drinks and low-pH beverages in recent years[1]. Too much soft drink drinking can erode the enamel of teeth. It has been suggested that soft drinks can be supplemented with ions such calcium, fluoride, and phosphate that are important for the development of hydroxyapatite in order to lessen their erosive potential [2].One of the most common dental diseases is dental erosion [3]. The chemical loss of mineralized tissue brought on by acid dissolving in the absence of bacteria is called erosion. Acids and tooth surfaces coming into frequent contact leads to demineralization via erosion [4].One of the most popular materials for treating and preventing tooth erosion lesions is fluoride [5]. Dental products containing calcium and phosphate have been shown to have an anti-erosive impact by promoting tooth remineralization [6]. Fluoride is one of the most electronegative ions, which quickly interacts with calcium to form calcium fluoride (CaF2). Mouth rinses used for remineralization usually contain either fluoride or calcium alone [3].Fluoride has

been the subject of preparations that mix it with various chemical agents to increase its efficiency and remineralize caries in its early stages. These preparations have become more widely available in recent years. Calcium glycerophosphate (CaGP) is one such substance. CaGP has been added to toothpastes, usually in conjunction with sodium monofluorphosphate, after its antibacterial qualities were demonstrated by studies [7]. An organic calcium phosphate salt with anti-cariogenic properties is called CaGP. It is hypothesised that CaGP increases the quantities of calcium and phosphate in plaque and in the hydroxyapatite in enamel, hence increasing plaque pH buffering and strengthening the enamel [8]. Calcium glycerophosphate (CaGP) has been reported to lessen enamel's acid dissolution [9]. The mineral loss and surface microhardness of enamel exposed to carbonated beverages were assessed by Manaswini et al. They came to the conclusion that adding CaGP to the carbonated drinks considerably stopped mineral loss and the deterioration of enamel's surface microhardness [10].

The remineralization impact of CaGP in a fluoride mouthwash on eroded permanent enamel is currently the subject of few studies. Thus, the objective of this in vitro investigation was to assess how a fluoride mouthwash containing CaGP affected the surface microhardness (SMH) of permanently damaged enamel.

2. Materials and method

After the removing of radicular part of each tooth, 48 sound-extracted premolars with sound enamel were collected, cleaned, and preserved in regular saline. Cracked teeth, diseases, and discoloration were not included. The specimens were positioned in acrylic resin that self-cured. The specimens were wet grounded in order to create a smooth, clean surface on the labial surfaces. A Vickers indenter (FM-700e Type D, Future-tech, Tokyo) was used to measure the baseline microhardness of the sound enamel on the labial surface. The indenter was used with a force of 100 g for 15 seconds. Every step of the test resulted in four indentations on each specimen. The % recovery of micro hardness (MH) was determined using the average MH.

2.1. Erosion creation

For twelve cycles, the samples were submerged in room temperature Coca-Cola for ten seconds, followed by fake saliva for ten seconds. Three times, the process was carried out at 6-hour intervals. The specimens were kept in artificial saliva between tests. Following the erosion process, deionized water was used to rinse the specimens, and they were then blotted dry. Solutions for demineralization (D) and remineralization (R) were made. Saliva was subjected to apatitic mineral supersaturation simulation using D and R solutions.

2.2. Procedure

Following the erosion procedure, the specimens were randomly assigned to four groups (n = 10). The groups' teeth were treated as follows: Group A received artificial saliva (no treatment); Group B received ammonia fluoride (1000 ppm fluoride from Amflor-Group Pharmaceuticals Ltd.); Group C received sodium monofluorophosphate (0.05% w/v) (Kedodent Mouthwash, Indoco Remedies Ltd.); and Group S received sodium monofluorophosphate + CaGP (SMFP+CaGP –224 ppmF+75 ppmCa) (Fluor Kin Mouthwash).Rinsing the teeth twice a day for duration of 12 days is known as the pH-cycling technique, which replicates the pH shift in the oral environment. Every tooth was incubated at 37 °C for three hours in D, two hours in R, and three hours in D. Using the Vickers indenter test procedure, the specimens' MH was determined following a 12-day pH cycling period.To test the antibiofilm action, *Streptococcus mutans* ATCC 25175 was treated for a whole day. Following a 24-hour incubation period, the diameter of the zones of inhibition (ZOI) was measured and the antibacterial efficacy was evaluated using the agar well diffusion technique. After being removed from the MRS broth, the sumba mare's milk was put into a test tube.One-way ANOVA was used in the statistical analysis of the collected data using SPSS software version 23.0.

3. Result

Table 1 displays the mean SMH values at baseline, during erosion, and following remineralization. There was no statistically significant difference in the groups' mean MH levels at baseline ($P = 0.435$). After the erosion process, the mean MH value fell from baseline and there were no significant differences among the groups ($P = 0.074$).The mean MH readings and %MHR in each group increased significantly following remineralization. On the other hand, the no treatment group's mean MH values and %MHR were the lowest. Additionally, $P < 0.001$ indicated that there were no significant variations in the mean MH value or %MHR across the three treatment groups.Groups B, C, and D showed a notable zone of inhibition against S. mutans. However, no inhibitory zone was seen in group A with control (Table 2).

4. Discussion

The purpose of this study was to determine how a fluoride mouthwash containing CaGP affects permanently damaged tooth enamel. In the current investigation, there was no discernible change in remineralization with or without CaGP. The remineralizing ability of the mouthwash with calcium and fluoride is higher than that of the fluoride mouthwash [3]. A substitute for achieving the effect of 1100 ppm F against demineralization was toothpaste with CaGP [11]. Using a pH cycling model, Emerenciano et al. assessed the effects of adding micrometric or nanometric [beta]-calcium glycerophosphate (β-CaGPm/β-CaGPn) to toothpastes containing 1100 ppm F on artificial enamel demineralization. They came to the conclusion that when coupled at the proper β-CaGP molar ratio, the fluoride toothpaste generated a greater effect [11]. The impact of adding calcium glycerophosphate (CaGP) to soft drinks on the deterioration of cow enamel was assessed by Barbosa et al. They came to the conclusion that adding CaGP to both drinks might be an alternate way to lessen their potential for erosive damage [1].

Table 1: Surface microhardness value (mean±SD) at baseline, after erosion, after remineralization, and the %MHR

Mouth rinse groups	Baseline	After erosion	Afterremineralization	%MHR	p
Group A-Control (Artificial saliva)	362.23 ± 3.12	312.11 ± 5.21	328.65 ± 4.45	18.53 ±6.45	<0.26
Group B-Amine fluoride (Amflor-Group pharmaceuticals Ltd)	364.28 ± 3.45	312.22 ± 6.13	352.12 ± 7.34	76.43 ± 12.65	<0.001*
Group C- sodium monofluorophosphate (0.05% w/v) (Kedodent Mouthwash, Indoco Remedies Ltd.),	363.31 ± 4.53	313.32 ± 8.43	362.65 ± 5.65	78.23 ± 26.56	<0.001*
Group D-SMFP+CaGP (Fluor Kin Mouthwash)	365.14 ± 4.46	310.04 ± 11.21	364.08 ± 6.23	85.54 ± 13.65	<0.001*
p	0.435	0.074	<0.001*	<0.001*	

Table 2: Zone of inhibition for *S. Mutans* among the groups

Groups	Mean±SD
Group A- Control group	00.00±0.00
Group B- Amflor mouth wash	10.25±0.23
Group C- sodium monofluorophosphate (0.05% w/v) (Kedodent Mouthwash, Indoco Remedies Ltd.)	18.35±0.63
Group D- SMFP+CaGP (Fluor Kin Mouthwash)	20.43±0.64

According to Puig-Silla et al., mouthwash containing both calcium and fluoride has a much higher capacity to remineralize teeth than mouthwash containing fluoride [7]. According to Carvalho et al., adding CaGP to fluoride varnishes did not increase the amount of fluoride bonded to enamel or improve the varnishes' ability to fend off early enamel degradation. Because calcium glycerophosphate (CaGP) interacts with hydroxylapatite to increase the latter's resistance to demineralization, it has been demonstrated to protect enamel from cariogenic demineralization [12]. Lynch et al used an in vitro bacterial flow cell model to study the anti-caries characteristics of calcium glycerophosphate (CaGP). They discovered that CaGP considerably decreased demineralization [13].Plaque buildup of calcium glycerophosphate can increase its ability to withstand bacterial acid assault by acting as a buffer [14]. Cavazan et al. came to the conclusion that CaGP interfered with dental caries and raised the concentrations of F, Ca, and P in the biofilm. It also caused the medium's pH to rise even after it was exposed to sucrose [15].According to research, calcium glycerophosphate made via a "green" method shows promise as a potential antibacterial agent against bacteria that live in the mouth [16]. Cavazana et al. [17] came to a similar conclusion as ours regarding the effects of CaGP, either alone or in combination with F, on bacterial cells. Additional research is required to verify the findings.

5. Conclusion

The effectiveness of fluoride mouth rinse in remineralizing eroded permanent enamel was comparable when it was used with or without CaGP. CaGP exhibited strong antibacterial activity.

Reference

[1] C.S. Barbosa, L.G. Montagnolli, M.T. Kato, F.C. Sampaio, M.A.R. Buzalaf. (2012). Calcium glycerophosphate supplemented to soft drinks reduces bovine enamel erosion. Journal of Applied Oral Science. 20: 410-413.

[2] T. Attin, K. Meyer, E. Hellwig, W. Buchalla, A.M. Lennon. (2003). Effect of mineral supplements to citric acid on enamel erosion. Archives of oral biology. 48(11): 753-759.

[3] P. Torsakul, P. Rirattanapong, W. Prapansilp, K. Vongsavan. (2023). Remineralization effect of calcium glycerophosphate in fluoride mouth rinse on eroded human enamel: An in vitro study. Journal of International Society of Preventive and Community Dentistry. 13(4): 327-332.

[4] D.C. Ruiz, L. Marqués Martínez, E. García Miralles. (2023). Dental Erosion and Diet in Young Children and Adolescents: A Systematic Review. Applied Sciences. 13(6): 3519.

[5] M. Epple, J. Enax, F. Meyer. (2022). Prevention of Caries and Dental Erosion by Fluorides—A Critical Discussion Based on Physico-Chemical Data and Principles. Dentistry Journal. 10(1): 6.

[6] M. Colombo, A. Dagna, G. Moroni, M. Chiesa, C. Poggio, G. Pietrocola. (2019). Effect of different protective agents on enamel erosion: An in vitro investigation. Journal of clinical and experimental dentistry. 11(2): e113.

[7] M. Puig Silla, J.M. Almerich Silla. (2009). Comparison of the remineralizing effect of a sodium fluoride mouthrinse versus a sodium monofluorophosphate and calcium mouthrinse: An in vitro study.

[8] R. Lynch. (2004). Calcium glycerophosphate and caries: a review of the literature. International dental journal. 54(S5): 310-314.

[9] T. Grenby, J. Bull. (1980). Use of high-performance liquid chromatography techniques to study the protection of hydroxylapatite by fluoride and glycerophosphate against demineralization in vitro. Caries Research. 14(4): 221-232.

[10] Y.H. Manaswini, K. Uloopi, C. Vinay, R. Chandrasekhar, K.S. RojaRamya. (2020). Impact of Calcium Glycerophosphate-supplemented Carbonated Beverages in Reducing Mineral Loss from the Enamel Surface. International Journal of Clinical Pediatric Dentistry. 13(1): 1.

[11] N.G. Emerenciano, A.C.B. Delbem, F.M.C. Gonçalves, E.R. de Camargo, F.N. de Souza Neto, L.F. Gorup, Y.T.C. Silva-Sousa, M. Danelon. (2024). Effect of nanometric β-calcium glycerophosphate supplementation in conventional toothpaste on enamel demineralization: An in vitro study. Journal of the mechanical behavior of biomedical materials. 151: 106354. Journal of the Mechanical Behavior of Biomedical Materials. 151: 106354

[12] T.S. Carvalho, M. Bönecker, M.J. Altenburger, M.A. Buzalaf, F.C. Sampaio, A. Lussi. (2015). Fluoride varnishes containing calcium glycerophosphate: fluoride uptake and the effect on in vitro enamel erosion. Clinical oral investigations. 19: 1429-1436.

[13] R. Lynch, J. Ten Cate. (2006). Effect of calcium glycerophosphate on demineralization in an in vitro biofilm model. Caries Research. 40(2): 142-147.

[14] F. Meyer, B.T. Amaechi, H.-O. Fabritius, J. Enax. (2018). Overview of calcium phosphates used in biomimetic oral care. The open dentistry journal. 12: 406.

[15] T.P. Cavazana, T.Y. Hosida, C. Sampaio, L.A. de Morais, D.R. Monteiro, J.P. Pessan, A.C.B. Delbem. (2021). Calcium glycerophosphate and fluoride affect the pH and inorganic composition of dual-species biofilms of Streptococcus mutans and Candida albicans. Journal of Dentistry. 115: 103844.

[16] J.A. Souza, D.B. Barbosa, A.A. Berretta, J.G. Do Amaral, L.F. Gorup, F.N. de Souza Neto, R.A. Fernandes, G.L. Fernandes, E.R. Camargo, A.M. Agostinho. (2018). Green synthesis of silver nanoparticles combined to calcium glycerophosphate: antimicrobial and antibiofilm activities. Future microbiology. 13(3): 345-357.

[17] T.P. Cavazana, T.Y. Hosida, C. Sampaio, L.A. de Morais, D.R. Monteiro, J.P. Pessan, A.C.B. Delbem. (2023). The Activity of Calcium Glycerophosphate and Fluoride against Cariogenic Biofilms of Streptococcus mutans and Candida albicans Formed In Vitro. Antibiotics. 12(2): 422.