Effect of acidic center-filled chewing gums on enamel microhardness: *In vitro* study

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ABSTRACT

Altered dietary habits are becoming etiological factor in the development of dental erosion. Evaluation of changes in microhardness of enamel after exposure to acidic center-filled chewing gums. Exposure of enamel to center filling (concentrated acid) and outer coating along with center filling in artificial saliva (dilute acid) of two chewing gums (Center Fresh and Bubbaloo) to evaluate the difference in microhardness change between primary and permanent enamel, on exposure to concentrated and dilute acids and two chewing gums. A total of 120 primary and permanent molar extracted teeth were painted with acid resistant varnish except a small buccal window and divided into eight groups according to type of dentition, chewing gum and acid exposure. 5 min acid exposures were done twice a day for 5 days. Then, specimens were submitted for microhardness studies. Independent sample t -test and paired t-test were used in statistical analysis. Statistically significant difference in reduction of microhardness is seen between groups exposed to dilute and concentrated acid, reduction of microhardness is more with dilute acids than concentrated acids.; but the difference is seen neither on exposure to two chewing gums nor between primary and permanent enamel. (1) Dilute acid causes a definite reduction in microhardness than concentrated acid. (2) Two types of chewing gums are equally effective. (3) Permanent and primary teeth are equally affected. (4) Outer coating of center-filled chewing gum is not protective against reduction in microhardness of enamel. Therefore, acidic center-filled chewing gums cannot be suggested to children.

Key words: Enamel microhardness, Center-filled chewing gums, Dental erosion

Access this article online

Website: www.jpediatrdent.org

DOI: 10.4103/2321-6646.151846

Quick Response Code:

INTRODUCTION

In the recent years, dental erosion has become a very common sign and is observed with increasing frequency in children and adults. The altered dietary habits, one of the consequences of modern lifestyle have to be taken into account when considering the augmented dental erosion status.[1]

Deciduous enamel and dentin are thinner than permanent teeth. Erosive process reaches dentin earlier in the deciduous enamel when compared with permanent enamel. The different susceptibility to erosion of permanent and primary enamel might increase over time and/or with decreasing pH of the acid.[2]

Despite the advantages of chewing gums, as being used as a delivery vehicle for substances such as calcium, bicarbonate, carbamide, chlorhexidine, fluoride and xylitol to improve oral health and reduce caries,[3] they also cause detrimental effects. The detrimental effects range from erosion to gastrointestinal disturbances.

Chewing gums are very popular among children due to their pleasant taste and their coloring effect in the mouth.[4] The aim of this study was to evaluate *in vitro* the changes in the microhardness of primary and permanent enamel with exposure to acidic center-filled chewing gum, the influence of outer coating of chewing gum along with center filling on permanent and primary enamel and differences of susceptibility of primary and permanent enamel to changes in microhardness with exposure to two center-filled chewing gums.

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Aim

The study aims to evaluate the demineralization caused by the acidic center-filled chewing gum on primary and permanent enamel.

Hypothesis

Reduction of microhardness is more with dilute acids when compared to concentrated acids because of their low surface tension, and this increases with an increase in its' frequency of application or usage.

MATERIALS AND METHODS

The present study was carried out in the Department of Pedodontics and Preventive Dentistry, Panineeya Maha Vidyalaya Institute of Dental Sciences, PMVIDS, Hyderabad in collaboration with Defence Metallurgical Research Laboratory, DMRL, Hyderabad, Andhra Pradesh, India. The study comprised of 60 primary and 60 permanent molars extracted because of orthodontic reasons, periodontal reasons and preshedding mobility. They were free of hypoplasia, hypo calcification, and cracks.

The extracted teeth were washed thoroughly under running tap water to remove blood, saliva and other debris. Teeth were cleaned with slurry of pumice and stored in deionized water till the experiment was started.

Artificial saliva was prepared in the department of biochemistry with the composition of 2 g of methyl-p-hydroxybenzoate, 10 g of sodium carboxymethyl cellulose, 0.625 g of potassium chloride, KCl, 0.059 g of magnesium chloride, $MgCl_26H_2O$, 0.166 g Calcium chloride, $CaCl_22H_2O$, 0.804 g of potassium hydrogen phosphate unhydrous, K_2HPO_4, 0.326 g of Potassium dihydrogen phosphate unhydrous, KH_2PO_4. The components were measured with common balance and added to 1 L of distilled water. Fluoride of 0.022 ppm was added to this solution. pH was checked with electronic digital meter and was 6.75.[5]

Concentrated acid was prepared by scooping out the center filling of the chewing gum and Dilute acid was prepared by mashing each chewing gum (center filling along with outer coating) in 5 ml of artificial saliva.

Study design

Extracted teeth were examined under stereo microscope to rule out the presence of cracks and all the surfaces of the teeth were painted with acid resistant varnish except a small window over buccal surface.

Groups are divided according to

1. Primary (D) and permanent (P) teeth
2. Type of acidic center-filled chewing gum (Center Fresh[1] and Bubbaloo[2])
3. Concentrated (a, center filling) and Dilute acid (b, mashed whole of chewing gum with artificial saliva) exposure as D1a, D1b, D2a, D2b, P1a, P1b, P2a and P2b.

Acid (concentrated or dilute) exposures of all 8 groups were done for 5 min at room temperature twice a day at 10 am and 1pm for 5 days [Figures 1-6]. After each exposure specimens were washed in deionized water for 20 s and immersed in artificial saliva at 37°C until the next experimental step. Artificial saliva was changed daily. After exposure, sectioning of specimens was done buccolingually through the window with an Buehler isomet low speed saw (Buehler's company).

Specimens were subjected to microhardness tests with knoop diamond indenter with 50 g load for 10 s at exposed and unexposed areas after mounting all the specimens in cold cure acrylic with a cut surface exposed [Figure 7].

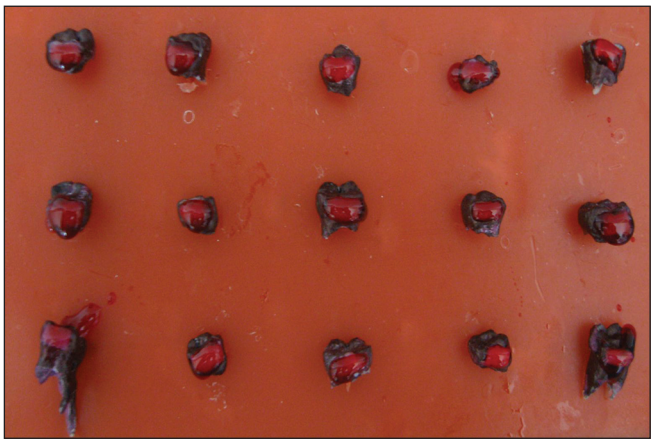


Figure 1: Center filling of bubbaloo-on primary enamel

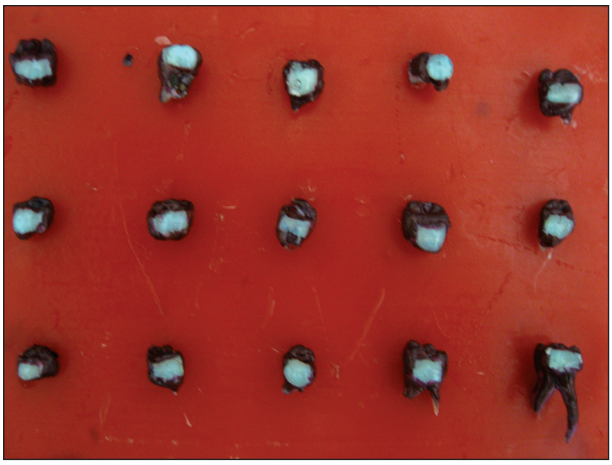


Figure 2: Center filling of Center Fresh on primary enamel

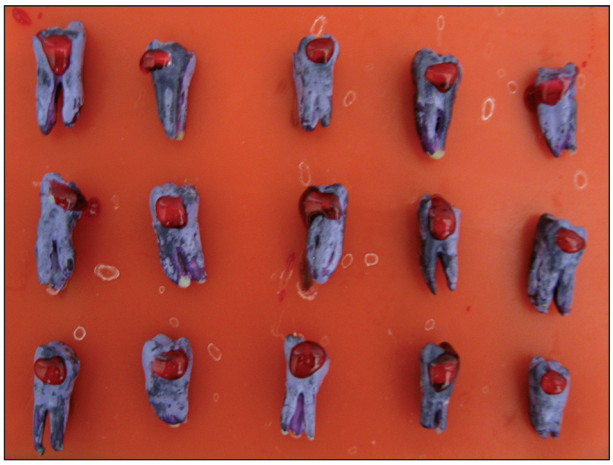


Figure 3: Center filling of bubbaloo on permanent enamel

Figure 4: Center filling of Center Fresh on permanent enamel

Figure 5: Exposure to mashed Bubbaloo in artificial saliva (dilute acid)

Figure 6: Exposure to mashed Center Fresh in artificial saliva (dilute acid)

The results were tabulated and subjected to statistical analysis using Microsoft Excel software (VWR International, Radnor, PA).

Statistics

Independent sample *t*-test has been used to:

1. Compare the erosive effect of (changes in means of Knoop microhardness values of unexposed and exposed areas exposed to) concentrated and dilute acids (*n* = 15) [Table 1 and Graph 1];
2. Comparison of erosive effect of [difference of means of Knoop microhardness values of unexposed and exposed primary and permanent teeth to] 2 chewing gums Center Fresh and Bubbaloo (*n* = 15) [Table 2 and Graph 2];
3. Comparison of the erosive effect on [difference between mean Knoop microhardness values of unexposed and exposed areas] primary and permanent enamel exposed to two center-filled chewing gums (*n* = 15) [Table 3 and Graph 3].

Paired *t*-test is used to:

4. Compare means of Knoop microhardness of exposed and unexposed surfaces in each group [*n* = 15] [Table 4].

RESULTS

The difference of reduction in microhardness between dilute and concentrated acid exposures was more with dilute acid than concentrated acid.

(*P* = 0.001 between D1a and D1b; 0.001 between D2a and D2b; 0.076 between P1a and P1b; 0.001 between P2a and P2b [comparison between concentrated, a and dilute acid, b]) [Table 1 and Graph 1].

There was no statistical difference between the reduction of microhardness exposed to two types of chewing gums (Center Fresh, 1 and Bubbaloo, 2) (*P* = 0.955 between D1a and D2a; 0.98 between D1b and D2b; 0.503 between P1a and P2a; 0.087 between P1b and P2b) [Table 2 and Graph 2].

There were no statistical difference between the reduction of microhardness of deciduous (D) and permanent (P) dentitions when exposed to concentrated and dilute acids (*P* = 0.4 between D1a and P1a; 0.835 between D2a and P2a; 0.082 between D1b and P1b; 0.835 between D2b and P2b) [Table 3 and Graph 3].

DISCUSSION

One of the consequences of a modern lifestyle is altered eating habits. These altered habits have various effects on overall health including dental health. Though dental caries is the most common dental health problem, other dental lesions such as dental erosion are becoming increasingly important. It has been a neglected problem because of unawareness of their causative factors and lack of immediate severe morbidity.

Table 1: Comparison between changes in means of Knoop microhardness values of unexposed and exposed areas exposed to concentrated and dilute acids (*n* = 15) (independent sample *t*-test)

Subgroup	Acid	Mean of (unexposed – exposed) KHN (erosive effect)	SD	*P*
D1 Primary teeth Center Fresh	Concentrated acid	−25.35	64.09	0.001
	Dilute acid	49.84	34.43	
D2 Primary teeth Bubbaloo	Concentrated acid	−26.62	56.56	0.001
	Dilute acid	50.29	58.37	
P1 Permanent teeth Center Fresh	Concentrated acid	−6.31	57.86	0.076
	Dilute acid	26.36	36.94	
P2 Permanent teeth Bubbaloo	Concentrated acid	−21.84	67.10	0.001
	Dilute acid	54.42	48.93	

SD: Standard deviation, KHN: Knoop hardness number

Increase in the consumption of soft drinks and chewing gums have led to the augmented prevalence of erosion. Acidic center-filled chewing gums have proven to be erosive[6] in nature and are being used by children more frequently. In our study, we have selected two most commonly used center-filled chewing gums, Center Fresh from Perfetti Van Melle (Mehrauli, Gurgaon, India) and Bubbaloo from Cadbury Adams (Matsonford Road, P.O. Box 6660, Radnor, PA) companies and evaluated and compared their erosive effect on primary and permanent enamel *in vitro*.

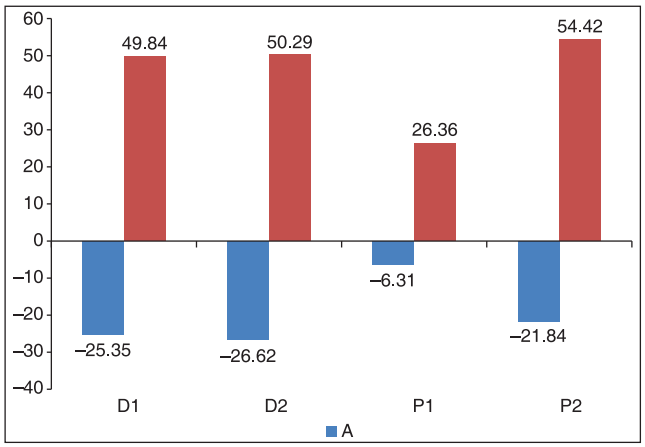
As children start using chewing gums and erosive drinks independently during the school going mixed dentition stage and chewing gums are also being used by adolescents to prevent stress, we did this study on both primary and permanent enamel. To get statistically significant results we have collected 60 primary and 60 permanent extracted teeth because of periodontal, orthodontic reasons and preshedding mobility.

Table 2: Comparison of means of Knoop microhardness values of unexposed and exposed primary and permanent teeth to 2 chewing gums Center Fresh and Bubbaloo (*n* = 15)

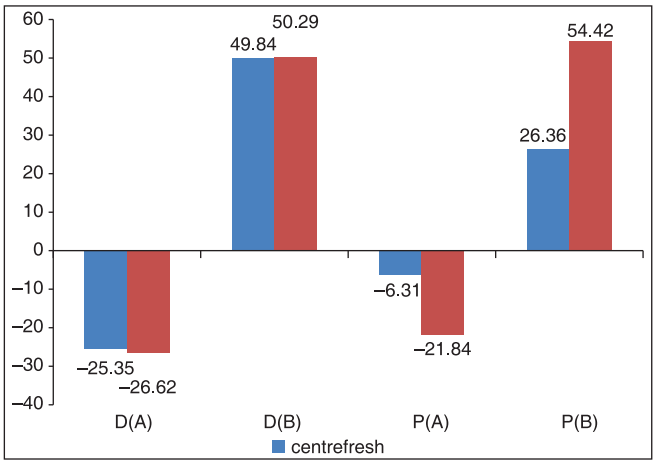
Dentition	Acid	Type	Mean of (unexposed – exposed) KHN (erosive effect)	SD	*P*
Deciduous teeth	Concentrated acid	1.00 Center Fresh	−25.35	64.09	0.955
		2.00 Bubbaloo	−26.62	56.56	
	Dilute acid	1.00 Center Fresh	49.84	34.43	0.98
		2.00 Bubbaloo	50.29	58.37	
Permanent teeth	Concentrated acid	1.00 Center Fresh	−6.31	57.86	0.503
		2.00 Bubbaloo	−21.84	67.10	
	Dilute acid	1.00 Center Fresh	26.36	36.94	0.087
		2.00 Bubbaloo	54.42	48.93	

SD: Standard deviation, KHN: Knoop hardness number

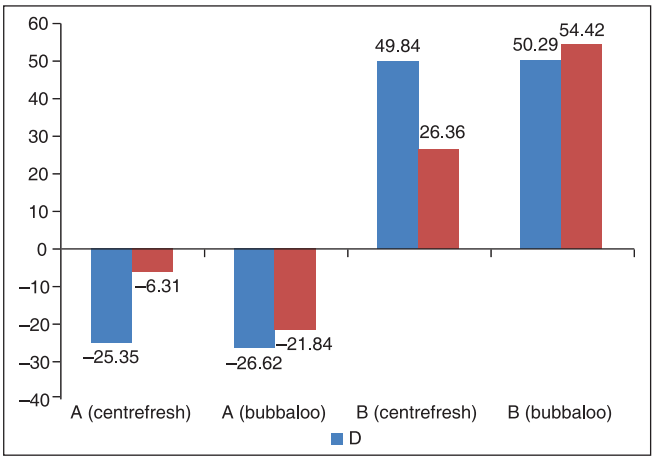
Figure 7: Knoop microhardness indentations



Graph 1: Comparison of difference in mean microhardness values of unexposed and exposed areas exposed to concentrated and dilute acids



Graph 2: Comparison of erosive effect of (diff erence in mean Knoop microhardness values of unexposed and exposed areas exposed to) Center Fresh and Bubbaloo chewing gums



Graph 3: Comparison of erosive effect (difference in mean Knoop microhardness values of unexposed and exposed areas) between deciduous and permanent teeth

Table 3: Comparison of mean Knoop microhardness values of unexposed and exposed areas between primary and permanent enamel to two types of center filled chewing gums (*n* = 15)

Acid	Type	Dentition	Mean of (unexposed — exposed) KHN (erosive effect)	SD	*P*
Concentrated acid	1.00 Center Fresh	Deciduous teeth	−25.35	64.09	0.4
		Permanent teeth	−6.31	57.86	
	2.00 Bubbaloo	Deciduous teeth	−26.62	56.56	0.835
		Permanent teeth	−21.84	67.10	
Dilute acid	1.00 Center Fresh	Deciduous teeth	49.84	34.43	0.082
		Permanent teeth	26.36	36.94	
	2.00 Bubbaloo	Deciduous teeth	50.29	58.37	0.835
		Permanent teeth	54.42	48.93	

SD: Standard deviation, KHN: Knoop hardness number

Deionized water has been used in many studies as a storage medium for extracted teeth.[7] In the present study, the teeth were stored in deionized water after washing in running water, till experiment was started.

All teeth were evaluated under Stereo microscope. Teeth with cracks, hypoplasia and caries were discarded. In many studies acid resistant varnish was used to isolate the area of acid exposure.[8] In our study, also to compare the effect of chewing gums between exposed enamel and unexposed enamel of the same tooth, teeth were painted with acid resistant varnish except for a window on the buccal surface.[9]

Both primary and permanent teeth were exposed to center filling (concentrated acid) [Figures 1-4] and resultant solution of milling each of the whole chewing gum (center filling and outer coating) [Figures 5 and 6] in 5 ml of artificial saliva. In this study, we evaluated the influence of center filling and the influence of outer coating of the chewing gum on the effect of center filling on the enamel. Therefore, we included the outer coating of the chewing gum and the center filling with artificial saliva in the preparation of dilute acid. Concentrated acid was prepared by scooping out the center filling of the chewing gum.

Chewing gum was exposed to acid resistant varnish uncovered area (window) for 5 days, twice a day. A demineralization treatment of 5 min is representative of the effects of acidic beverage consumption. Although longer acid exposure times has been reported in the range of 10-60 min and shorter acid exposure times in the range of 1-4 min. Thus it was considered that a 5 min exposure time would give an overall appropriate level of *in vitro* erosion severity.[10] In our study we have exposed the specimens to the contents of the chewing gum for 5 min.

As it is known that a repeated application of demineralization cycle leads to a more severe damage of enamel apatite, which cannot be recovered even after an exposure to remineralizing solutions for several days. In the literature, studies have shown that acidic exposure for 5 days caused erosive effect.[2,11] In our study, we have exposed the buccal window of enamel to the contents of chewing gum for 5 days twice a day.

In our study, we have used artificial saliva to simulate oral conditions and to compare the effect of chewing gum on teeth *in vivo*. Amount of stimulated saliva secreted per minute is 1 ml/min, and considering that child chews

Table 4: Comparison between means of Knoop micro hardness of exposed and unexposed surfaces in each group (*n* = 15) (paired *t*-test)

Group	Hardness	Mean	SD	*P*
D1a Deciduous teeth Center Fresh Concentrated acid	Exposed	250.58	29.03	0.148
	Unexposed	225.22	62.00	
D1b Deciduous teeth Center Fresh Dilute acid	Exposed	221.37	20.72	<0.001
	Unexposed	271.22	31.50	
D2a Deciduous teeth Bubbaloo Concentrated acid	Exposed	256.62	50.87	0.09
	Unexposed	230.00	67.21	
D2b Deciduous teeth Bubbaloo Dilute acid	Exposed	223.69	41.21	0.005
	Unexposed	273.97	48.94	
P1a Permanent teeth Center Fresh Concentrated acid	Exposed	264.46	49.12	0.679
	Unexposed	258.15	43.78	
P1b Permanent teeth Center Fresh Dilute acid	Exposed	270.33	36.82	0.015
	Unexposed	296.69	20.62	
P2a Permanent teeth Bubbaloo Concentrated acid	Exposed	283.09	53.15	0.228
	Unexposed	261.22	38.71	
P2b Permanent teeth Bubbaloo Dilute acid	Exposed	223.99	68.51	0.001
	Unexposed	278.42	38.24	

SD: Standard deviation

chewing gum approximately for 5 min, we prepared dilute acid by milling one chewing gum with 5 ml of artificial saliva, in the preparation of dilute acid. To simulate the oral conditions, during the experimental period, we stored the specimens in artificial saliva after exposing the teeth to the concentrated and dilute acids of chewing gum. Artificial saliva was changed every 24 h. After the completion of 5 days, experimental procedure all specimens were removed from artificial saliva and stored in deionized water. This is to prevent remineralization by artificial saliva of demineralized enamel.

In our study, to compare microhardness of enamel on both exposed and unexposed areas of the same tooth we made cut sections of the tooth through the buccal window of enamel and microhardness was analyzed on both the surfaces. In the literature, there are studies in which cut sections were performed by diamond disc and slow speed diamond grit blades of Isomet of Buehler Company.[12] In our study, we made cut sections with slow speed diamond grit blades of Isomet of Buehler Company.

In the literature, many studies have mounted the specimens in cold cure acrylic.[13,14] In our study, too, cut sections of specimens were mounted in cold cure acrylic with a cut section exposed for a flat surface, to facilitate microhardness study. In the previous studies knoop diamond indentations were made with 50 g load for 10 s.[15,16] In our study too 3 knoop micro hardness indentations with 50 g load for 10 s were taken at subsurface on both exposed and unexposed areas of enamel [Figure 7] and the mean value is calculated.

Many studies have shown that there is a significant reduction in enamel's microhardness under acidic stuffing challenge.[15-19] In our study, there is a significant reduction in enamel's microhardness with the exposure to dilute acid and no significant reduction with the exposure to concentrated acid.

In a study done by Bolan *et al*.,[6] on erosive effects of acidic center-filled chewing gum on primary and permanent enamel, it is found that for more concentrated acid, the stuffing is viscous creating a higher surface tension and a higher contact angle with the enamel surface, presenting a lower flow and lower dental erosion. A higher change in enamel's superficial hardness is seen when the diluted acid was used. It is due to a lower surface tension which promoted higher flow and consequently a lower contact angle with the enamel's surface and higher dental erosion. In our study also effect of dilute acid in decreasing enamel's microhardness was more than concentrated acid (center filling of the chewing gum).

In the results, mean Knoop microhardness values of exposed area are found to be more than unexposed areas exposed to concentrated acid with no statistically significant change or reduction in microhardness. This can be attributed to higher viscosity or surface tension of concentrated acid and remineralizing capability of artificial saliva.[20,21] In a study done by Bolan *et al*.[7] on acidic center-filled chewing gums, they have used vibrator during exposure to concentrated acid. In the present study, for practical reasons we could not use a vibrator. This also can be attributed to lower penetration of acid into the enamel.

The mean Knoop microhardness values of exposed area are found to be less than unexposed areas exposed to dilute acid with statistically significant change or reduction in microhardness. This can be attributed to greater penetration capacity or lower surface tension of dilute acid.[6,20,22,23]

In some studies, they found that erosion is different for deciduous teeth compared to permanent teeth[24,25] and was no significant difference in erosion between deciduous teeth and permanent teeth.[23] However in our study, there was no statistically significant reduction

in microhardness of enamel between primary and permanent enamel.

In our study, the evaluation and comparison of the effect of the decrease in microhardness values of enamel between Center Fresh and Bubbaloo chewing gums, we found no statistically significant difference.

In our study, as we have intended to evaluate the influence of outer coating on the reduction of microhardness of enamel, we prepared dilute acid by milling the whole chewing gum in 5 ml artificial saliva and exposed to enamel surface. It has reduced the microhardness values significantly. It indicates that chewing gum still possesses erosive effect and the outer coating does not provide any protective action against dental erosion.

CONCLUSION

1. There is a definite reduction in microhardness between unexposed and exposed areas in groups exposed to dilute acids. There is no significant reduction in microhardness in groups exposed to concentrated acids.
2. The dilute acid has got more definite effect of reducing microhardness of sound enamel when compared to concentrated acids, indicating its effect *in vivo* as the child chews the gum by combining it with saliva.
3. Two types of chewing gums, Center Fresh and Bubbaloo are equally effective in reducing microhardness of enamel.
4. Both permanent and primary teeth are equally effected by reduction in microhardness after exposure to center-filled chewing gums.
5. The role of outer coating of center-filled chewing gum is not protective against reduction in microhardness of enamel, and these chewing gums cannot be suggested to children.

ACKNOWLEDGMENTS

I would like to thank Dr. Karunakar, MDS, Princial, Panineeya Mahavidyalaya Institute of Dental Sciences and Research Center, Hyderabad, without whom this dissertation would not have been possible.

My sincere thanks to Dr. Prameela Bhupathi Raju MDS, Reader for her timely advice and guidance.

I express my sincere gratitude to Dr. D. Mahesh Kumar MDS, Reader for his valuable guidance and support.

Dr. M. Shantan Reddy MDS and Dr. Gayatri Moghe MDS, senior lecturers for their encouragement, guidance and support.

I am highly obliged and express my sincere gratitude to Dr. V. Bhagirath, MDS, Head, Department of Oral Pathology for his help in the preparation of artificial saliva. I also acknowledge the help given by Mr. Siva Kumar, Histo Pathologist, Department of Oral Pathology, Panineeya Mahavidyalaya Institute of Dental Sciences and Research Center, Hyderabad.

I express my deep sense of gratitude to Dr. M. Srinivas, Scientist G, Defence Metallurgical Research Laboratory (DMRL), Hyderabad for his help and the facilities he offered to me in DMRL. I acknowledge the help given by Mr. M. Sai Madhav, senior technical assistant, DMRL, in the Stereo microscopic study and Microhardness study.

I would like to thank my colleagues Dr. D. Arthi, Dr. P. Shailaja, Dr. Priyanka, Dr. Harshini and Dr. Rabecca for their cooperation, help and logistical support.

I would express my heartful thanks to my beloved husband P. Vasudevan and my daughter Amuktha Malyada for their help and cooperation given during this study.

Most importantly, I would like to thank my parents, Dr. M. S. Ramanuja charyulu and Dr. Mrs. B. K. Ranga and brother, Dr. M. Vijaya Saradhi and sister-in-law Dr. G. Sujatha for their unconditional support and love.

I pray Almighty for giving me strength and perseverance in completion of this study.

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How to cite this article: Mudumba VL, Radhika M, Srinivas N. Effect of acidic center-filled chewing gums on enamel microhardness: *In vitro* study. J Pediatr Dent 2015;3:24-31.

Source of Support: Nil. **Conflict of Interest:** None declared.