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Assessment of Acidogenicity of Commercially Available Biscuits on Salivary pH - An *in vivo* Study

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Authors' contributions

This work was carried out in collaboration between both authors. Author BVB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JP managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Saliva is a complex secretion consisting of 99% of water and remaining 1% of organic and inorganic molecules. Sucrose and starches are the predominant dietary carbohydrates in modern societies. Among all the foods consumed by children, chocolates and biscuits are the most common. Therefore this present in vivo study was conducted to assess the acidogenic effect of commercially available biscuits on salivary pH among 10 to 15 years old children. Study Design used in the study was In Vivo clinical study (Pilot Trail). The population collected in the survey was children between the age group of 10 - 15 years old children. 4 Groups were considered and 10 in each group. Group 1: Hide and Seek, Group 2: Good Day, Group 3: Dream and Cream, Group 4: Oreo. Sampling method used in the study was conducted as simple random sampling. Ethical approval of the study was obtained from Saveetha Institutional Review Board. Informed consent of the children were obtained from the parents. Descriptive statistics were expressed by means of mean and standard deviation. Shapiro Wilks test used to test the normality of the data set. Kruskal Wallis test was used to find the difference in mean Salivary pH between the groups and within the

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groups at Baseline, Immediate and after 15 min, 30 mins. A statistically significant difference in mean Salivary pH was observed between the groups at Immediate and after 30 mins ($p<0.05$). The mean Salivary pH was significantly dropped in Oreo, Dream cream and Hide & Seek groups at various time-periods. Based on the results of the present study, it can be concluded that maximum drop in mean salivary pH was observed in Group IV followed by Group II and Group I. It was observed that in all the groups, the pH gradually got back to near normal levels due to the buffering mechanism of saliva.

Keywords: Biscuit; children; salivary; pH; Acidogenicity.

1. INTRODUCTION

Oral health is defined as "being free of chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal (gum) disease, tooth decay and tooth loss, and other diseases and disorders that affect the mouth and oral cavity" [1,2]. Saliva has a pH normal range of 6.2-7.6 with 6.7 being the average pH. Resting pH of mouth does not fall below 6.3. In the oral cavity, the pH is maintained near neutrality (6.7-7.3) by saliva. The saliva contributes to maintenance of the pH by two mechanisms [3]. Saliva is a complex secretion which contains 99% of water and 1% of organic and inorganic molecules. The diverse functions of saliva in the oral tissues are mastication, deglutition, taste sensation, speech and initial digestion of the carbohydrates. This would be impossible without the salivary secretions. The interface between the saliva and oral tissue is the site of many dynamic reactions which affect both the soft tissues and hard tissues of the oral cavity. Saliva provides the physiologic environment in the oral cavity where the complex interactions between the agent, host and the environment factor occur, to bring about demineralization of the tooth and subsequent development of caries. The salivary parameters that affects the stability of enamel in the oral environment are pH of saliva, salivary flow rate, oral clearance, concentrations of calcium, phosphate and fluoride ions and salivary levels of the oral microorganisms [4].

However, in case of dietary substances, the pH alone cannot be a predictive of potential of any acidic food stuff or beverages to cause erosion, as other chemical factors like adhesion and chelating properties, pKa values, calcium, phosphate, and fluoride content, behavioral factors like eating and drinking habits, excessive consumption of acids, lifestyle and biological factors like composition of saliva, flow rate, buffering capacity, dental and soft tissue anatomy, pellicle formation, dental and soft tissue anatomy, tooth composition, etc., modify the erosive process [5].

Among many other causes influencing dental caries, diet has long been acknowledged as a major cause [6]. Snacks and bakery foodstuffs like biscuits are the most preferred food items in the present decade. Thus, it is important to know its carcinogenicity. The food items such as cream sandwich, cookies and potato chips are more likely to be retained on teeth in larger amounts than foods like milk chocolate, caramels, and jelly beans. These food particles retained on the tooth surface are further hydrolyzed by microorganisms which lead to further decrease in pH of saliva [7].

Increasing prevalence of childhood obesity is seen globally. According to a study conducted by Gupta N, et,al. in India, 22.0% children are suffering from childhood obesity [8]. One of the major causes for this high prevalence is the change in lifestyle and dietary patterns [9]. Some dietary patterns appear quite common among children and adolescents such as snacking, usually on energy-dense foods; meal skipping, particularly breakfast or irregular meals; wide use of fast food; and low consumption of fruits and vegetables [10,1].

Diet affects the integrity of the teeth; quantity, pH, and composition of the saliva; and plaque pH. Sugars and other fermentable carbohydrates, after being hydrolyzed by salivary amylase, provide substrate for the actions of oral bacteria, which in turn lower plaque and salivary pH [11]. The pH at which demineralization occurs is referred to as the critical pH and is approximately 5.5. The resultant low pH favors the growth of the acidogenic and aciduric bacteria (mutans streptococci) [12]. We have successfully completed numerous epidemiological and clinical studies for the betterment of our community [13–19]. Therefore the aim of the present study is to assess the acidogenic effect of commercially

available biscuits on salivary pH among 10 to 15 years old children.

2. MATERIALS AND METHODS

2.1 Study Design

The present research was an invivo study carried out to evaluate the changes in salivary pH, after consumption of commercially available biscuits in the Indian market.

2.2 Sample Size and Study Population

The total sample size was 40. Children in the age group between 10-15 years old were enrolled in this present study.

2.2.1 Inclusion criteria

Subjects who were caries free i.e. DMFT score =0

2.2.2 Exclusion criteria

Subjects who were using any medication at the time of study or in period of last 15 days prior to the study and subjects who were suffering from any systemic illness were excluded from the study. All the study subjects were similar with respect to their dietary habits, oral hygiene measures and other lifestyle factors which could have a significant effect on the study results.

2.3 Study Groups

Group I: Hide and Seek (n=10)
Group II: Good Day (n=10)
Group III: Dream and Cream (n=10)
Group IV: Oreo (n=10)

2.4 Study Procedure

Unstimulated salivary samples were collected for each study subject at least one hour after their breakfast. The saliva was collected from the participants in a sterile bottle and Baseline salivary pH was estimated (before consumption of biscuits) (Fig. 1). The pH strip was used to estimate the salivary pH (Fig. 2). Biscuits were given to the participants and and then unstimulated saliva samples were collected at the following fixed time intervals:

1) 1st follow up - immediately after test food consumption
2) 2nd follow up - 15 mins after the test food consumption
3) 3rd follow up - 30 mins after the test food consumption

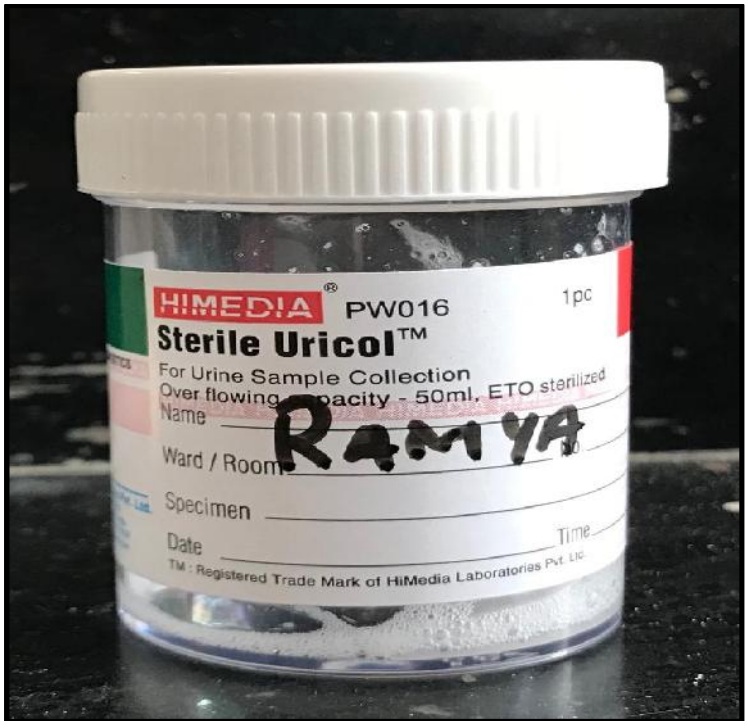

Fig. 1. Saliva collection using sterile container (Uricol)

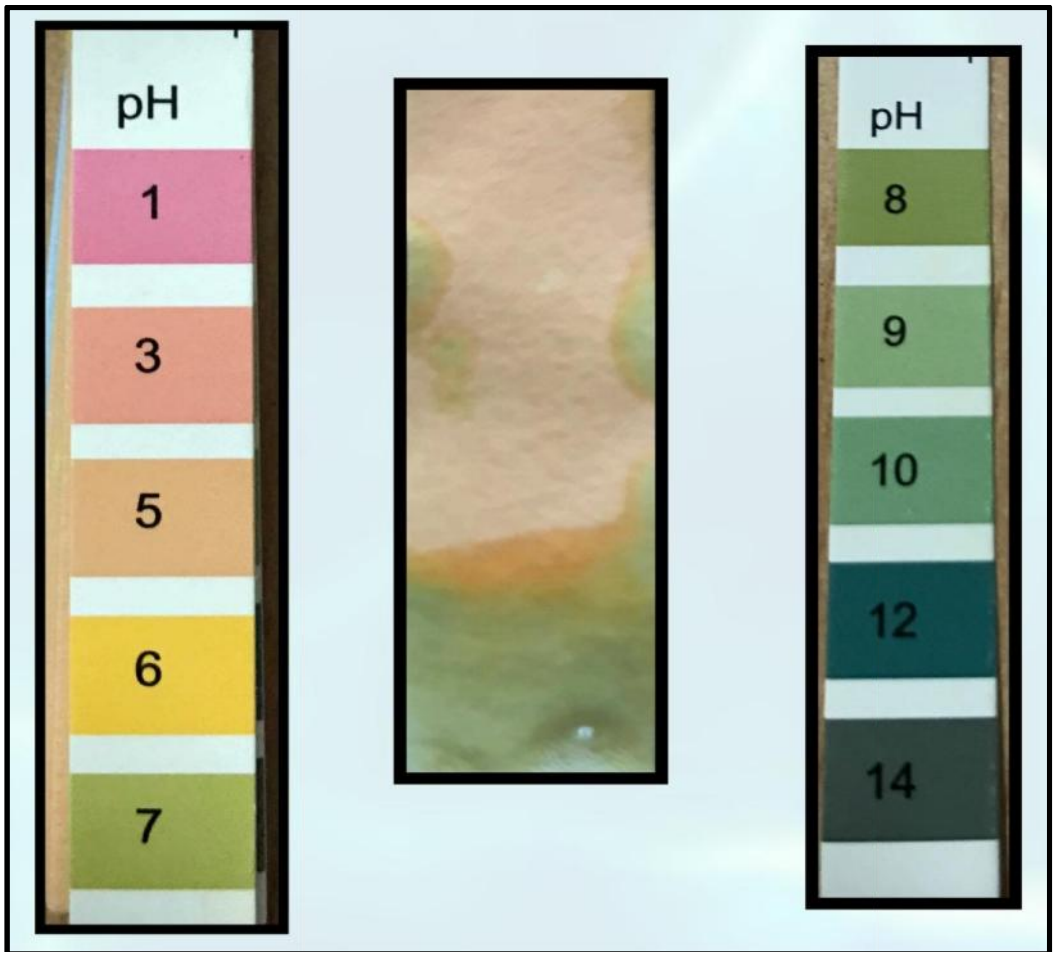

Fig. 2. Assessment of salivary pH using pH strip

2.5 Collection of Salivary Samples

For the collection of unstimulated saliva, subjects were seated comfortably, with their eyes open in a standard dental chair. The subjects sat with their head bent forward and spat into a sterile container. 1 ml of unstimulated saliva was collected at baseline and at each time interval after Biscuit consumption (Fig. 1).

2.6 Statistical Analysis

The data was entered in the Microsoft Excel Sheet and analyzed using the IBM SPSS Version 20.0 statistical packageArmonk, NY: IBM. Corp). Numerical data were presented as mean and standard deviation values. For the test, a p value of <0.05 is to be considered statistically significant. Shapiro Wilks test used to test the normality of the data set. Kruskal Wallis test was used to find the difference in mean Salivary pH between the groups and within the groups at Baseline, Immediate and after 15min, 30 mins.

3. RESULTS AND DISCUSSION

Saliva plays a multiple role in the oral cavity. The diverse functions of the oral tissues are mastication, deglutition, taste sensation, speech and initial digestion of the carbohydrates would be impossible without the salivary secretions. It is a complex biological fluid that it is practically impossible to replicate from individual components. In a healthy state, the pH of saliva is maintained usually between 6.7 to 7.4. Salivary buffering capacity and sugar clearance are important dynamic effects of saliva which prevent demineralization of tooth structure [20].

The study was carried out on 40 subjects with the age of 10 to 15 year, selected among the patients visiting Saveetha Dental College and Hospital, Chennai. Many factors play a major role in the change in salivary pH after consumption of any food item. When a food is consumed, an admixture of saliva and food is formed. There is

an increased flow of saliva, as a result of food consumption, leads to an increase in pH of saliva but the overall change depends on the sugar content, intrinsic pH, buffering capacity and manner in which the food is consumed [21]. According to the study conducted by Konig et al. observed that food consumption lowers salivary pH, and that this drop is followed by a rise in pH [22].

Table 1 shows the comparison of Mean salivary pH between the Groups at different time periods. Kruskal Wallis test was used to find the difference between the groups at Baseline, Immediate and after 15min, 30 mins. A statistically significant difference in mean Salivary pH was observed between the groups at Immediate and after 30 mins ($p<0.05$). The mean Salivary pH was significantly dropped in Oreo, Dream cream and Hide & Seek groups at various time-periods. The comparison of mean Salivary pH in Group I at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean Salivary pH between time periods for Group I (Hide& Seek) and was found to be statistically significant [Kruskal Wallis test value- 9.58; p-0.02($p<0.05$)]. Hence proving, there is a significant drop in mean Salivary pH from baseline (refer Fig. 3). Fig. 4 shows the comparison of mean Salivary pH in Group II at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean Salivary pH between time periods for Group II (Good day) and was found to be statistically significant [Kruskal Wallis test value- 9.81; p-0.02($p<0.05$)]. Hence proving, there is a significant drop in mean Salivary pH from baseline. Fig. 5 represents the comparison of mean Salivary pH in Group III at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean Salivary pH between time periods for Group III (Dream Cream) and was found to be statistically highly significant [Kruskal Wallis test value- 11.91; p-0.008($p<0.05$)]. Hence proving, there is a significant drop in mean Salivary pH from baseline. The comparison of mean Salivary pH in Group IV at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean Salivary pH between time periods for Group IV (Oreo) and was found to be statistically highly significant [Kruskal Wallis test value- 12.13; p-0.007($p<0.05$)]. Hence proving, there is a significant drop in mean Salivary pH from baseline (refer Fig. 6)

Table 1. Depicts the comparison of mean salivary pH between the groups at different time periods. Kruskal Wallis test was used to find the difference between the groups at Baseline, Immediate and after 15min, 30 mins. A statistically significant difference in mean salivary pH was observed between the groups at Immediate and after 30 mins ($p<0.05$). The mean Salivary pH was significantly dropped in Oreo, Dream cream and Hide & Seek groups at various time-periods

Time Periods		Salivary pH		Kruskal Wallis test	P value
		Mean	SD		
Base Line	Hide and seek	7.20	0.12	0.32	0.95
	Good day	7.22	0.05		
	Dream cream	7.23	0.06		
	Oreo	7.23	0.06		
Immediate	Hide and seek	6.73	0.22	8.10	0.04*
	Good day	6.91	0.20		
	Dream cream	6.63	0.22		
	Oreo	6.53	0.21		
15mins	Hide and seek	6.85	0.26	5.46	0.14
	Good day	7.03	0.12		
	Dream cream	6.75	0.26		
	Oreo	6.69	0.14		
30 Min	Hide and seek	6.94	0.22	11.10	0.01**
	Good day	7.12	0.10		
	Dream cream	6.78	0.16		
	Oreo	6.60	0.18		

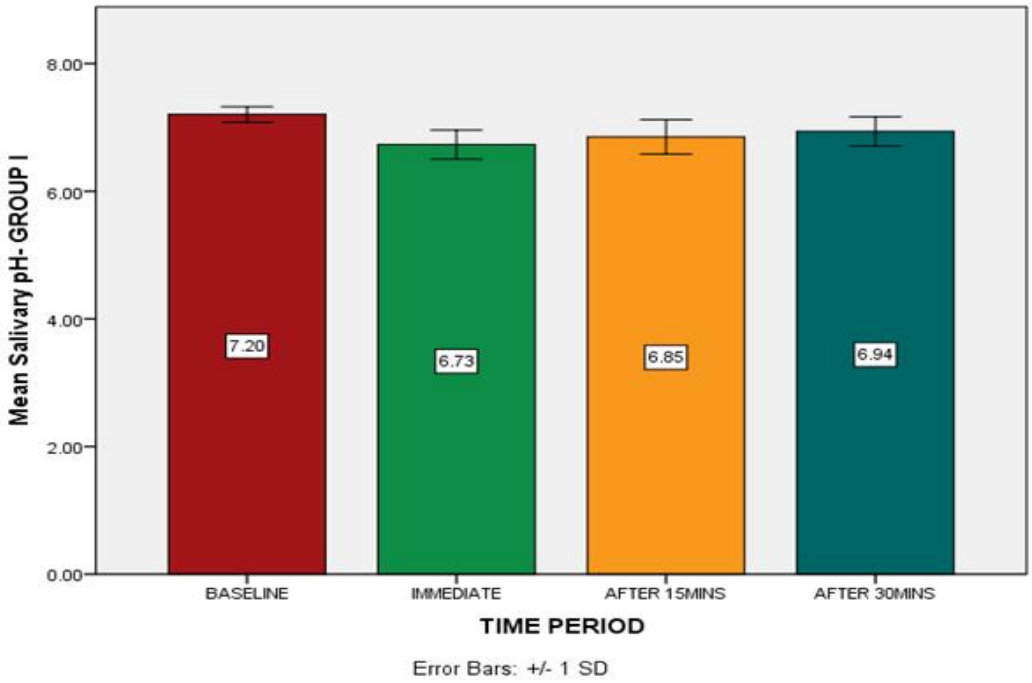

Fig. 3. Depicts the comparison of mean salivary pH in Group I at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean Salivary pH between time periods for Group I (Hide& Seek) and was found to be statistically significant [Kruskal Wallis test value- 9.58; p-0.02 (p<0.05)]. Hence proving, there is a significant drop in mean salivary pH from baseline

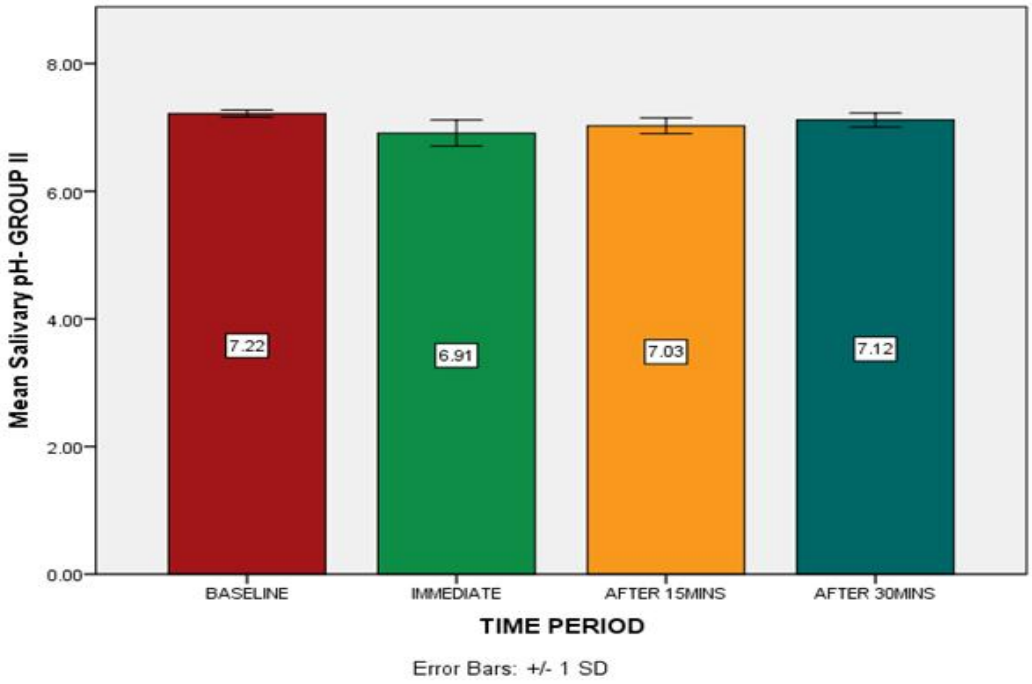

Fig. 4. Depicts the comparison of mean salivary pH in Group II at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean salivary pH between time periods for Group II (Good day) and was found to be statistically significant [Kruskal Wallis test value- 9.81; p-0.02 (p<0.05)]. Hence proving, there is a significant drop in mean salivary pH from baseline

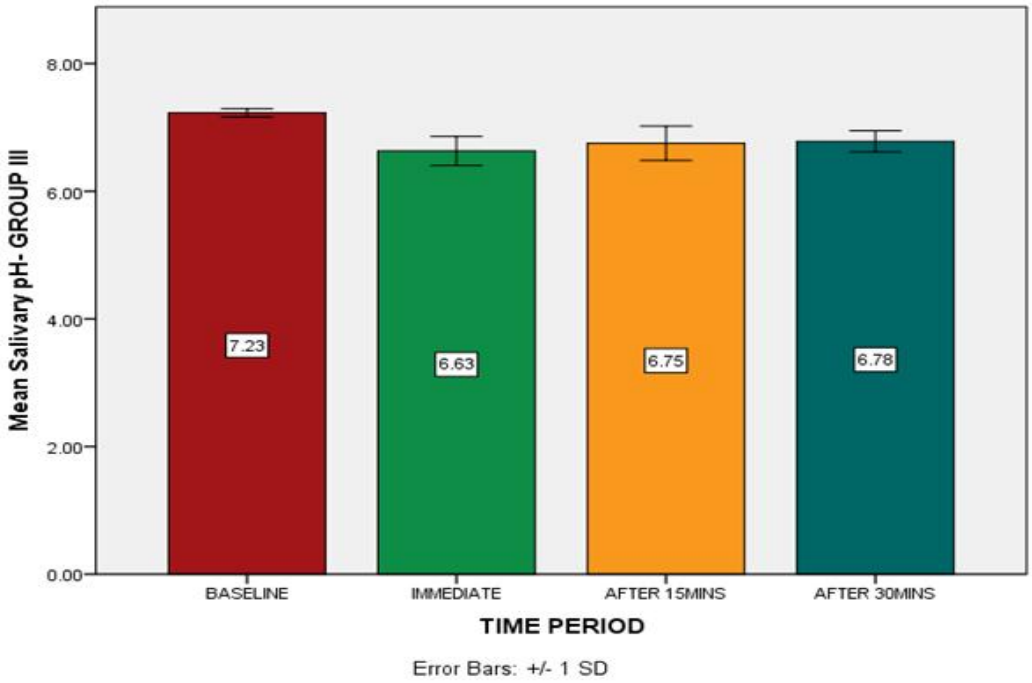

Fig. 5. Depicts the comparison of mean salivary pH in Group III at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean salivary pH between time periods for Group III (Dream Cream) and was found to be statistically highly significant [Kruskal Wallis test value- 11.91; p-0.008 (p<0.05)]. Hence proving, there is a significant drop in mean salivary pH from baseline

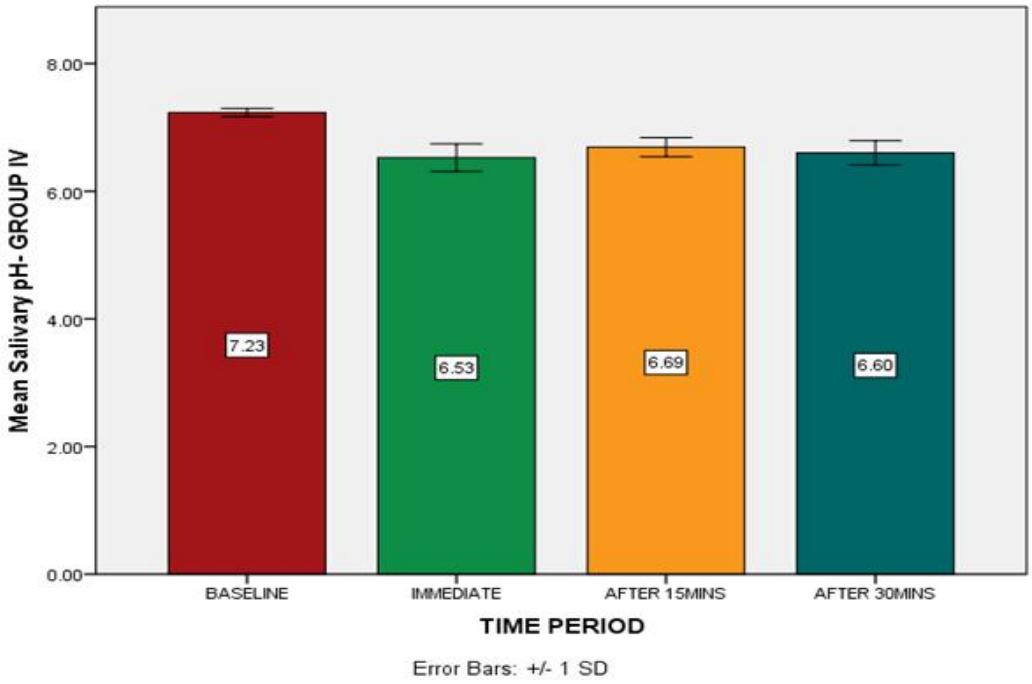

Fig. 6. Depicts the comparison of mean salivary pH in Group IV at different time periods (Baseline, Immediate and after 15min, 30 mins). Kruskal Wallis test was used to find the difference in mean salivary pH between time periods for Group IV (Oreo) and was found to be statistically highly significant [Kruskal Wallis test value- 12.13; p-0.007 (p<0.05)]. Hence proving, there is a significant drop in mean salivary pH from baseline

4. CONCLUSION

Based on the results of the present study, it can be concluded that maximum drop in mean salivary pH was observed in Group IV followed by Group II and Group I. It was observed that in all the groups, the pH gradually got back to near normal levels due to the buffering mechanism of saliva.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Ethical approval of the study was obtained from Saveetha Institutional Review Board. Before the start of the study, the purpose and methodology of the study was explained and written informed consent were obtained from the parents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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