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Original Article

Dentistry Section

Efficacy of Probiotic and Herbal Chewing Gums on Salivary pH in 6-11 Years Age Group Children: A Randomised Clinical Trial

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ABSTRACT

Introduction: Saliva is a crucial diagnostic tool to evaluate oral health. Chewing sugar-free gum is an effective method to increase salivary flow and can also be employed a medium for the administration of medicinal ingredients.

Aim: To evaluate and compare the effect of chewing xylitol, herbal and probiotic chewing gums on salivary acidogenicity levels.

Materials and Methods: A single centre, randomised, double-blind, multiple arm parallel clinical trial was conducted on 60 children aged between 6-11 years. Baseline salivary pH was recorded with the pH meter and then the participants were asked to consume chocolate and salivary pH was recorded at an interval of 5, 30, and 60 minutes. Then children were randomly allocated to Group A-Xylitol chewing gum (control group, n=20), Group B-Herbal chewing gum (n=20), and Group C-Probiotic chewing gum (n=20) and instructed to chew gum according to their assigned group and salivary pH was again recorded at an interval of 5, 30, and 60 minutes. The change in salivary pH was evaluated using one-way Analysis of Variance (ANOVA) and Post-hoc Bonferroni's test.

Results: The mean age of the study population was 9.23±1.38 years. Statistically significant ($p<0.001$) increase in pH was recorded post consumption of probiotic, xylitol and herbal chewing gums and the mean salivary pH values recorded at 60 minutes were 7.20±0.21, 7.01±0.23 and 6.43±0.22, respectively.

Conclusion: The observations of the study showed that xylitol, herbal and probiotic chewing gums reversed the fall in salivary pH, after an acidogenic challenge with probiotic being better among the three groups.

Keywords: Dental caries, Oral health, Preventive dentistry, Remineralising agent

INTRODUCTION

Dental caries stands out as a leading chronic lifestyle disease. The interaction between substrate and oral microorganisms in the microenvironment provided by the host is the cause of the disease. This multifactorial nature of the disease complicates its preventive strategies in all age groups [1]. According to Wincour E et al., (2001), among the preadolescent and adolescent groups chewing usage is a popular practise. It is a superior caries preventative method in children [2]. Xylitol has been actively used as a component of gums owing to its caries preventing actions like reduction in overall plaque volume and reduction in oral bacterial load [3]. Comparable results were reported with the use of herbal mastic gums. The anti-inflammatory and antioxidant properties of this gum are primarily responsible for subsiding gum inflammations caused by plaque accumulation and thereby secondarily reduce the cariogenic activity [4]. A recent modification that comprises the inclusion of probiotics in chewing gums has shown results comparable and even superior to xylitol-based chewing gums. Probiotics are known to alter the microenvironment in which the substrate breakdown by oral bacteria occurs. This helps in limiting cell to cell adhesion and thereafter plaque accumulation on the tooth surface [5]. Regulatory agencies such as the World Dental Federation (2015), the United Kingdom Oral Health Foundation (2018), and numerous other national dental organisations throughout the world acknowledge and endorse the oral health benefits of chewing sugar-free gums [6]. The literature [7,8] on mastic gum (Pistacia Lentiscus Linn tree) and probiotic gum (*Bacillus coagulans*) are scarce.

Since there are no similar studies carried out before to assess the effect of Bacillus coagulans and mastic chewing gums on salivary pH levels in children. Hence, the present study was conducted with aim to assess and compare the effects of xylitol, herbal, and probiotic-based commercially available chewing gums on salivary acidogenicity levels in children.

MATERIALS AND METHODS

This was a single centre double-blind randomised multiple arm parallel clinical trial conducted on 60 subjects of both genders aged 6-11 years from Primary and Higher Secondary Schools of Raipur city, Chhattisgarh, India from 29th October 2021 to 13th December 2021.

The research protocol was approved by the Ethical Committee of Maitri College of Dentistry and Research Centre (MCDRC/2021/OCT/580). The present research was conducted in accordance with the Declaration of Helsinki as revised in 2013. The trial was registered with the Primary Registries in the World Health Organisation (WHO) Registry Network (CTRI/2021/10/037683). The consent was obtained from school authorities prior to the commencement of the study.

Inclusion criteria:

- Children within the age range of 6-11 years.
- Children with dental caries score of dmft/DMFT <3.
- Children with no history of medication in the past three months.
- Children willing to participate.

Exclusion criteria:

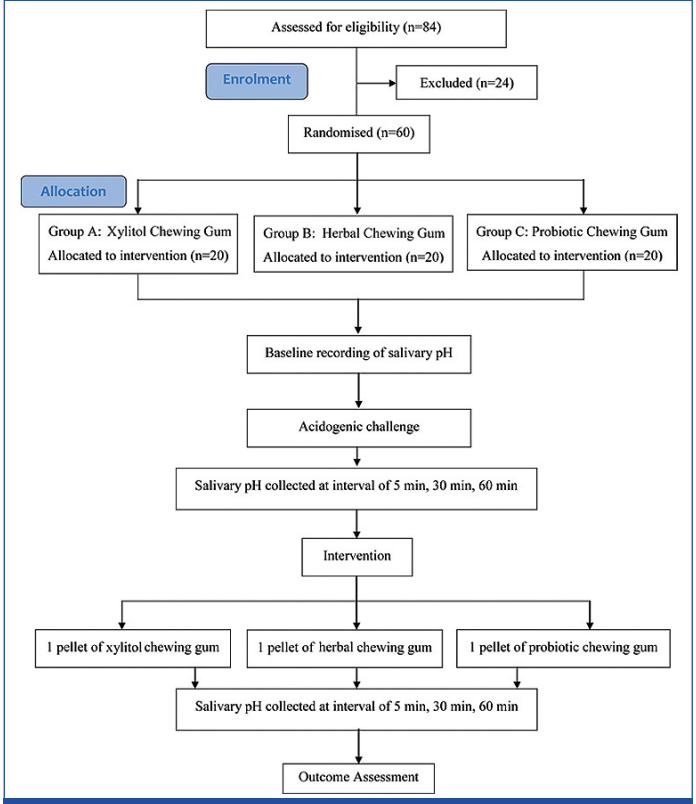
- Medically compromised children
- Children with history of systemic allergies and diseases.
- Children using any prosthetic/orthodontic appliance.

To limit the bias in the present study, the age, dietary habits, oral hygiene measures and time of the day for the collection of all the samples from participants were standardised.

Sample size calculation: G*Power (Version 3.1.9.2, Released in 2014, Kiel University, Germany) was used to estimate the sample size. Sample size estimation was done at 0.05 significance level and at 80% power of the study, with an effect size of 0.50 [9], the sample size obtained was 20 in each group.

Blinding, Randomisation and Allocation Concealment

Enrolled participants in all three arms and study coordinators were blinded to study group. All three chewing gums were provided as identically labelled white packing precoded by the study coordinators intermixed in the box [Table/Fig-1].



[Table/Fig-1]: Flow Diagram in accordance with Consolidated Standards of Reporting Trials (CONSORT) Statement.

Saliva Collection

1. **Unstimulated saliva sample:** The baseline pH value was determined by taking unstimulated saliva samples from all 60 subjects. The children were refrained from intake of any food or beverage (water exempted) 30 minutes prior the saliva collection. The baseline pH of saliva was measured after collecting 5 mL of unstimulated saliva from each subject in a sterile plastic container.

2. **Stimulated saliva sample post acidogenic challenge:** After eating a chocolate (Cadbury 5-Star Bar- 16 g), the children's saliva was collected at 5, 30, 60 minutes intervals to determine the salivary pH.

3. **Stimulated saliva sample post chewing gum consumption:** Then participants were thereafter, divided into the following three groups:

- Group A (n=20): Xylitol chewing gum: Trident Bubble Gum (control)
- Group B (n=20): Herbal chewing gum: Herbaveda-Gum Mastic
- Group C (n=20): Probiotic chewing gum: BonAyu Mouth Dissolving Strips

The children were asked to chew one pellet of chewing gum of their allocated group each respectively for 15 minutes. After spitting out the chewing gum and properly expectorating the children's saliva, sample was collected at 5, 30, 60 minutes intervals to determine the salivary pH.

Estimation of Salivary pH

Hannah digital pH metre (HI98127 pHep®4 pH/Temperature Tester with 0.1 pH resolution) was used to record the pH of both unstimulated and stimulated saliva. The pH meter's bulb was dipped in a plastic container containing saliva to record the salivary pH, after which the pH values were displayed digitally on the pH meter's body.

The outcome variables are change in salivary pH on consumption of chewing gum post acidogenic challenge.

STATISTICAL ANALYSIS

Statistical Package for Social Science software (SPSS) (Version 24.0, Chicago, IL) was used for data analysis. Intragroup comparison of the means of salivary pH levels for all the groups were done using Repeated measures ANOVA and intergroup comparison was done using Post-hoc Bonferroni's test. The level of significance was set at $p<0.05$.

RESULTS

A total of 60 children were enrolled with mean age of group A, B and C as 9.80±2.46, 9.40±2.39 and 8.90±2.32 years, respectively. In our study male participants in group A, B and C were 5 (25%), 18 (90%), 16 (80%) and females were 15 (75%), 2 (10%), 4 (20%) respectively. The mean dmft of group A, B and C was found to be 2.29±1.1, 2.41±1.4 and 2.31±0.8, respectively but the difference of dmft among three groups were statistically insignificant.

The mean pH of unstimulated saliva for group A, B, and C was 7.29±0.21,7.03±0.24 and 7.11±0.18, respectively [Table/Fig-2]. There was significant reduction in the salivary pH levels after consumption of chocolate in all the groups at 60 minutes (p=0.001), [Table/Fig-3]. On comparison of mean pH of the chewing gum stimulated saliva in three groups at 30 minutes, the mean pH was highest in group C followed by group B then group A [Table/Fig-4].

Groups	pH of unstimulated saliva (Baseline values) (Mean±SD)	p-value#
Group A (Xylitol chewing gum)	7.29±0.21	Group A vs B=0.510 Group B vs C=0.310 Group A vs C=0.610
Group B (Herbal chewing gum)	7.03±0.24	
Group C (Probiotic chewing gum)	7.11±0.18	

[Table/Fig-2]: Mean pH scores of unstimulated saliva in children.
SD: Standard deviation, One way ANOVA applied, #Post-hoc Bonferroni's applied

Groups	Time intervals			
	5 minutes Mean±SD	30 minutes Mean±SD	60 mins Mean±SD	p-value
Group A	6.41±0.19	5.38±0.26	5.85±0.16	0.001*
Group B	6.30±0.22	5.31±0.21	5.73±0.24	0.001*
Group C	6.43±0.24	5.23±0.15	5.66±0.21	0.001*

[Table/Fig-3]: Comparison of mean pH scores of stimulated saliva at different time intervals after consumption of chocolate.
One way ANOVA applied, *Statistically significant p<0.05. SD: Standard deviation

Groups	5 minutes		30 minutes		60 minutes	
	Mean	SD	Mean	SD	Mean	SD
Group A	6.01	0.25	6.27	0.24	7.01	0.23
Group B	6.01	0.28	6.23	0.23	6.43	0.22
Group C	6.20	0.28	6.59	0.17	7.20	0.21
p-value	0.051		0.0001		0.0001	

[Table/Fig-4]: Mean pH of the chewing gum stimulated saliva in three groups.
One-way ANOVA applied, *p-value significant at p<0.05

On intergroup comparison, at 5 minutes the mean salivary pH scores between group A and group B showed no significant difference (p-value=1.00), whereas mean salivary pH values were significantly greater in group C compared to group A and B (p-values=0.028, 0.028, respectively). Group C showed significant increase in mean salivary pH values compared to group A and B (p-values-0.001,0.001 respectively) and insignificant difference was observed between group A and group B (p-value=1.00) at 30 minutes. There was significant increase in the mean salivary pH scores in between the groups at the end of 60 minutes [Table/Fig-5]. Intragroup comparison showed that the mean difference in pH scores was not significant in group A and group B from 5 minutes to 30 minutes, whereas

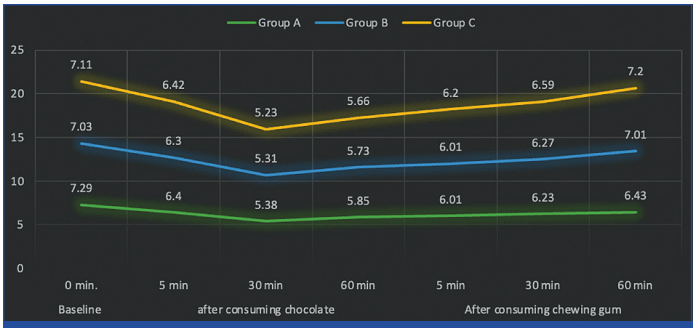
significant in group C (p-0.078,0.06 and 0.001, respectively). Mean difference in pH scores was significant in group A and group C and not significant in group B (p-value=0.001, 0.001 and 0.055, respectively) from 30 minutes to 60 minutes. The mean difference in group A and C was significant (p-value=0.0001) and less significant in group B (p-value=0.001) from 5 to 60 minutes compared to other groups [Table/Fig-6]. Salivary pH change from baseline to post consumption of chewing is demonstrated using [Table/Fig-7].

Time intervals	Groups	Mean±SD	p-value
5 mins	Group A vs Group B	6.01±0.25 vs 6.01±0.28	1.00
	Group A vs Group C	6.01±0.25 vs 6.20±0.28	0.028*
	Group B vs group C	6.01±0.28 vs 6.20±0.28	0.028*
30 mins	Group A vs Group B	6.27±0.24 vs 6.23±0.23	1.00
	Group A vs Group C	6.27±0.24 vs 6.59±0.17	0.001*
	Group B vs Group C	6.23±0.23 vs 6.59±0.17	0.001*
60 mins	Group A vs Group B	7.01±0.23 vs 6.43±0.22	0.001*
	Group A vs Group C	7.01±0.23 vs 7.20±0.21	0.024*
	Group B vs Group C	6.43±0.22 vs 7.20±0.21	0.001*

[Table/Fig-5]: Intergroup comparison of mean pH scores of saliva at 5 mins, 30 mins and 60 mins after consumption of chewing gum.
One-way ANOVA applied, *p-value significant at p<0.05

Groups	Time intervals	Mean pH	Mean difference	p-value
Group A (Xylitol chewing gum)	5 mins-30 mins	6.01-6.27	-0.26	0.078
	30 mins-60 mins	6.27-7.01	-0.74	0.0001*
	5 mins-60 mins	6.01-7.01	-1.00	0.0001*
Group B (Herbal chewing gum)	5 mins-30 mins	6.01-6.23	-0.22	0.06
	30 mins-60 mins	6.23-6.43	-0.20	0.055
	5 mins-60 mins	6.01-6.43	-0.42	0.001*
Group C (Probiotic chewing gum)	5 mins-30 mins	6.20-6.59	-0.39	0.001*
	30 mins-60 mins	6.59-7.20	-0.61	0.0001*
	5 mins-60 mins	6.20-7.20	-1.00	0.0001*

[Table/Fig-6]: Intragroup comparison of mean pH scores of saliva at different time intervals after consumption of chewing gum.
SD: Standard deviation, One-way ANOVA applied, *p-value significant at p<0.05



[Table/Fig-7]: Line Diagram for comparison of mean pH scores at different time intervals.

DISCUSSION

In present study, among all the chewing gums given post acidogenic challenge, probiotic chewing was found to be most effective followed by xylitol chewing gum and herbal chewing gum.

Children aged 6-11 years belonged to concrete operational period, hence, they understand the concept of rules and follow instructions properly [10]. Normal salivary pH is in the range of 6.2-7.6 [11]. In the present study, pH ranged between 6.7-7.6. Similar values for unstimulated pH were noted by Murthy GS et al., (2020) [11]. It was important to record the unstimulated pH for standardisation of participants and so that the later drop and rise in stimulated salivary pH could be compared to it. According to study, done by Dawes C (1969), the concentration of ions in the saliva keeps changing with time [12]. Hence, in the present study, saliva samples were taken at three different time intervals to record the variations in the salivary pH values after acidogenic challenge and consumption of chewing gums.

Acidogenic challenge in the form of chocolate was given to the participants prior to the consumption of chewing gums because it is known to produce acidogenic response in the saliva and plaque and demonstrate children regarding these harmful effects of chocolate. A sudden drop in salivary pH of similar pattern was clearly noted in all the groups. This is similar to the Stephan's curve which demonstrates the rapid fall in pH due to acid production by plaque bacteria on consumption of sugar [13]. In the present study, the pH noted at 30 minutes in all the subjects showed a fall beyond the critical pH (5.5), below which the enamel starts to demineralise [14]. However, recovery was made in the next 30 minutes when the pH, still lower than baseline and that after five minutes of chocolate consumption and is comparable with study conducted by Murthy GS et al., (2020) [11]. This pattern was again by Stephan's curve which elaborates that the buffering action of saliva gradually starts neutralising the acids [15,16].

Chewing gum has been shown to increase salivary flow and pH thereby, lowering the incidence of dental caries [17]. Xylitol is a naturally occurring 5-carbon sugar polyol that has been shown to reduce the incidence of dental caries by increasing salivary flow and pH, decreasing plaque formation and bacterial adherence (antimicrobial), inhibiting enamel demineralisation (reduces acid production), and having a direct inhibitory effect on MS [18,19]. According to a recent systematic review, the effect of xylitol on caries incidence in children was small, with very low-quality evidence from randomised controlled trials indicating that xylitol's protective efficacy is questionable [7]. As a result, the authors used herbal and probiotic chewing gums in this investigation to compare their efficacy with xylitol on salivary pH levels in children.

The present study used Herbaveda Gum Matic which consists of terpinen-4-ol and a-terpineol obtained from the leaves and stem of Pistacia Lentiscus Linn tree [8]. Aksoy A et al., (2006) [20] and Biria M et al., (2009) [8] reported that chewing mastic gum has been shown to reduce the MS count in saliva and enhance the pace of remineralisation of artificial carious lesions.

Probiotics play a pivotal role in creating better oral health by interfering with formation of biofilm as they compete with microbes for existing substances and produce substances that hinder the growth of microbes responsible in deteriorating oral hygiene [21]. Probiotic chewing gum used in the present study was BonAyu Probiotics Mouth Dissolving Strips. Each serving contains spore forming 1.0 billlion Colony-forming Unit (CFU) *Bacillus coagulans* (Lactobacillus sporogenes). Unlike the vegetative lactobacillus and Bifidobacterium spp. which cannot sustain harsh processes and also require refrigeration, the *B. coagulans* being a spore-forming probiotic strain sustains the harsh manufacturing procedures and can be easily incorporated in any dosage form without loss of viability [22,23].

Post acidogenic challenge, children in all three groups demonstrated a significant increase in salivary pH, five minutes after chewing gum consumption, followed by a steady increase at 30 and 60 minutes eventually reaching neutral salivary pH, though the pH values of saliva were still below than the baseline values (unstimulated saliva) except for children in Group C (probiotic chewing gum). This is in accordance with studies conducted by Kumar S et al., (2013) [24], Hegde RJ and Thakkar JB (2017) [25], and Shinde MR and Winnier J et al., (2020) [26] after chewing xylitol gums. A significant increase in the salivary pH levels in children, who consumed probiotic chewing gum was noted compared to xylitol and herbal chewing gum groups in the present study. Probiotics provides a suitable environment for tooth remineralisation by counteracting acidic conditions in the mouth and inhibiting the growth of cariogenic bacteria and increasing the salivary pH [27,28]. Probiotics inhibit the growth of carcinogenic bacteria by production of bactriocin and also buffers acidic pH of

S. No.	Author's name and year	Place of study	Number of subjects	Age of children	Objective	Conclusion
1	Aksoy A et al., [20], 2005	Turkey	25	20-30 years	Evaluate antibacterial activity of mastic chewing gum against *S. mutans*	They concluded that mastic gum had significant antibacterial activity against *S. mutans* and *mutans streptococci* and it may be a useful adjunct in caries prevention
2.	Karami-Nogourani M et al., [30], 2011	Isfahan, Iran	15	18-20 years	Compare the effect of five different flavors of sugar free chewing on properties of saliva	the spearmint and cinnamon-flavoured gum significantly increased the salivary pH.
3.	Kumar S et al., [24], 2013	Belgaum	30	10-12 years	To determine efficacy of sugared and sugar-free (xylitol) chewing gums in altering salivary properties.	They reported that there was a a marked increase in the pH of saliva and plaque in children consuming the sugar-free (xylitol) chewing gum.
4.	Biria M et al., [8], 2014	Tehran	45	20-30 years	Assess efficacy of three types of mastic gums on pH of the saliva.	The increase in salivary pH with the pure and xylitol mastic gum was insignificant.
5.	Naveena P et al., [31] 2015	Bangalore	150	5-8 years	Evaluate and compare the anticariogenic action of two commercial chewing gums Mastic and Xylitol on the salivary *Streptococcus Mutans* count.	Mastic chewing gum showed statistically significant reduction in the number of colonies as compared to Xylitol.
6.	Srivastava S et al., [21], 2016	Lucknow	60	20-25 years	Determine efficacy of probiotic curd on salivary pH.	There was a significant increase in salivary pH and reduction of salivary *S. mutans* counts upon usage of probiotic curd.
7.	Vantipalli UK et al., [32], 2017	Guntur district, Andhra Pradesh	4000	10-12 years	Determine effectiveness of chewing gums on salivary properties in children	Sugar-free gums have aids in declining caries incidence
8.	Hegde RJ and Thakkar JB, [25], 2017	Kharghar region, Navi Mumbai	60	8-12 years	Determine effectiveness of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and xylitol-containing chewing gums on salivary properties.	CPP-ACP chewing gum and xylitol gum can significantly increase the physiochemical properties of saliva.
9.	Padminee K et al., [33] 2018	Chennai	20	18-25 years	Evaluate the effectiveness of different chewing gums based on salivary properties.	Salivary properties were better in participants that consumed chewing containing CPP-ACP.
10.	Shinde MR and Winnier J, [26], 2020	Navi Mumbai	60	8-13 years	Determine efficacy of the different types of chewing gums on properties of saliva	Efficacy of stevia and xylitol gum were comparable in reducing salivary *S. mutans* counts.
11.	Ratna Sudha M et al., [23], 2021	Telangana	48	5-15 years	Determine effect of probiotic strain on salivary properties of children	Bacillus coagulans Unique IS2 (2 billion cfu) inhibits growth of *mutans streptococci* and lactobacilli.
12.	Present study	Raipur, Chhattisgarh	60	6-11 years	Efficacy of herbal, xylitol and probiotic chewing on salivary pH	All three chewing gums increased salivary pH and Probiotic was found to be best among three.

[Table/Fig-8]: Related studies to present research [8,20,21,23-26,30-33].

oral cavity. Bacteriocins inhibits the growth of bacteria by penetrating the surface of pathogenic bacteria, that leads to leakage of amino acids and inorganic salts from its cell thereby, killing the bacteria [29]. Similar studies to present research on efficacy of chewing gums are discussed in the [Table/Fig-8] [8,20,21,23-26,30-33].

Limitation(s)

The fact that, the authors did not standardise all of the participants' oral hygiene status could be a source of bias in the present study. This may have been avoided, if all of the participants had received oral prophylaxis. Studies with larger sample size and with a prolonged follow-up period need to be conducted in order to generalise results of present study.

CONCLUSION(S)

Chewing gum has been considered as a medium for the delivery of therapeutic ingredients and can be used as an adjuvant in the prevention of caries. Probiotics provides a suitable environment for tooth remineralisation by neutralising acidic conditions in the mouth and inhibiting the growth of cariogenic bacteria and increasing the salivary pH. Probiotic chewing gums produced the best outcomes, followed by xylitol and lastly herbal chewing gums. Probiotic chewing gums are easy, economic and efficient method of caries prevention in children.

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