ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

Efficacy of probiotic and green tea mouth rinse on salivary pH after a chocolate challenge

Dr. Shilpa Shetty Naik
Professor and Head, Department of Pediatric and Preventive dentistry, D.Y. Patil school of dentistry, Nerul, Navi Mumbai.
naik.dr.shilpa@gmail.com
Dr. Ashveeta Shetty
Senior lecturer, Department of Pediatric and Preventive dentistry, D.Y. Patil school of dentistry, Nerul, Navi Mumbai.
ashveeta.shetty@dypatil.edu
Dr. Sanjana Kodical
Lecturer, Department of Pedaitric and Preventive dentistry
DY Patil University School of Dentistry, Dr. D. Y. Patil Nerul, Navi Mumbai-
sanjana.kodical@dypatil.edu
Dr. Kajol Thakur
Post Graduate student, Department of Pedaitric and Preventive dentistry
D.Y. Patil school of dentistry, Nerul, Navi Mumbai.
mailkajol.thakur@gmail.com
Dr. Shagorika Choudhury
Post Graduate student, Department of Pedaitric and Preventive dentistry
D.Y. Patil school of dentistry, Nerul, Navi Mumbai.
shagorika.choudhury6@gmail.com
Dr. Nikitha Balasubramanian
Post Graduate student, Department of Pedaitric and Preventive dentistry
D.Y. Patil school of dentistry, Nerul, Navi Mumbai.
dr.nikithabala@gmail.com

Abstract:
Objectives: The aim of the study was to assess the efficacy of probiotic and green tea mouth rinse on salivary pH after a chocolate challenge. **Method:** The subjects were selected and randomly divided into 3 groups consisting of 15 children each: Group 1 (Probiotic mouth rinse), Group 2 (Green Tea mouth rinse) and Group 3 (Distilled water mouth rinse). After determining the resting salivary pH, each child is given a chocolate bar to eat followed by one of three interventions and the salivary pH is evaluated immediately after exposure, 5 minutes, after the intervention and sixty minutes later. One-way ANOVA and Tukey's post hoc test was used. Level of significance was set at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.
Result: The comparison of mean pH scores for green tea showed that the pH of saliva was increased in the children after rinsing with green tea (6.65 to 7.23) and was highly statistically significant ($p<0.001$). Similarly, when pre and post mean pH was compared in the probiotic group pH increased from 6.66 to 6.94 which were also highly statistically significant. Whereas in the distilled water group, the pH decreased from 6.57 to 6.48 **Conclusion:** Within limitations and based on the results

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

of this trial, we may conclude that both, green tea and probiotic mouth rinses were effective in reversing the salivary pH after a chocolate challenge and maintaining it for 60 minutes highly significantly.

INTRODUCTION:

Dental caries begins and progresses largely as a result of bacterial, dietary, and host response interactions.[1] Saliva is essential for maintaining good oral health and for establishing a suitable ecological balance as different salivary constituents have unique effects on the characteristics of saliva such as salivary flow, pH, and buffering capacity.[2] Saliva's pH, among several other factors, is crucial for preserving the health of the oral cavity.[3] Dental caries can occur most frequently in environments with an acidic pH. surface It is generally known that when the pH drops below the threshold pH of 5.5, enamel dissolves.[4] As the degree of saturation, the pH rises, it results in the remineralization of the tooth.

Even though the association of cariogenic diet and dental caries is well known, children and chocolates are often inseparable, hence, many affordable and effective agents have recently been tested in the combat against dental caries. Commercially available mouth rinses like chlorhexidine support mechanical plaque control, but search for alternative antiplaque drugs has been sparked by adverse effects such brown discoloration of the teeth and tongue, oral mucosal erosion, and taste disturbance.[3]

This emphasises the need to reverse the pH drop as soon as the exposure occurs by using adjuncts safe and easy to use for children. Incorporation of probiotics which are helpful microorganisms into these chemical adjuncts will help as, when given in sufficient proportions, they have positive effects on the overall health of the host whereas, Polyphenolic substances, such as those found in plant foods like green tea, have biological features that include antioxidants and anti-inflammatory actions.[4]

Therefore, the purpose of this study is to assess how green tea and probiotic mouthwashes affect pH levels.

METHODOLOGY:

This study included children attending the Out Patient Department of the Department of Pediatric and Preventive dentistry of D.Y. Patil school of dentistry, Nerul, Navi mumbai. The subjects were selected and randomly divided into 3 groups which consisted of 15 children each : Group 1 (Probiotic mouth rinse), Group 2 (Green Tea mouth rinse) and Group 3 (Distilled water mouth rinse). After determining the resting salivary pH, each child was given a chocolate bar (Cadbury Dairy Milk Chocolate bar, 13.2g) to eat followed by one of the three interventions and the salivary pH is evaluated immediately after exposure, five, twenty minutes after the intervention and sixty minutes later. Ethical clearance was obtained from the Institutional Ethical Committee.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

Inclusion criteria:

1. Healthy children aged between 4 - 12 years without any known systemic condition
2. Children with informed consent from their parents
3. Children with no active carious lesions
4. No history of use of antimicrobial agents or any other drugs (up to within 4 weeks).

Exclusion criteria:

1. Children with a known history of allergy to the mouth rinse used in the study or any of its components.
2. Children using any other commercially available probiotic products or oral hygiene aids other than tooth brushing.

Preparation of probiotic mouthrinse

Darolac, a commercially available probiotic product, was used to make a probiotic mouthrinse (Aristo Pharmaceuticals, India). It consists of 1.25 billion freeze-dried bacteria, including Lactobacillus acidophilus, Lactobacillus rhamnous, Bifidobacterium longum, and Saccharomyces boulardii, in 1 g of the powder. For usage as a mouthwash, the contents of the sachet were dissolved in 10 ml of distilled water.[6]

Preparation of green tea mouth rinse

Fresh green tea (Lipton green tea dip bags with the packaging date of less than one month ago)were used. 2 g of green tea dip bag were dipped in 100 ml of warm water for five minutes to make two percent green tea (10 ml for each participant).[7]

Salivary pH measurements

Prior to mouth rinsing, at baseline and post intervention, 2 cc of saliva samples were collected. Unstimulated saliva samples were collected by asking the patient to bend their heads forward and pooling saliva for 60 seconds and then spitting into a disposable container while sitting upright in a well-lit, well-ventilated environment.[8] To avoid any bias in the saliva concentration due to circadian rhythm, saliva was collected in the morning between 10.00 am and 10.30 am.[9] In order to reduce potential food debris and saliva stimulation, children were also instructed to refrain from eating or drinking anything (apart from water) 1 hour before to saliva collection.

Merck pH strips were used to measure the pH of saliva as per manufacturers. The pH value was recorded after 30 seconds of colour change and comparing it to a colour code chart.

The indicated mouth rinse was given to the appropriate groups after the baseline recording of salivary pH. They were told to swish the 10 ml of mouth rinse for 1 minute. The paired t-test was employed in the statistical analysis. Statistics were deemed significant at P 0.05.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

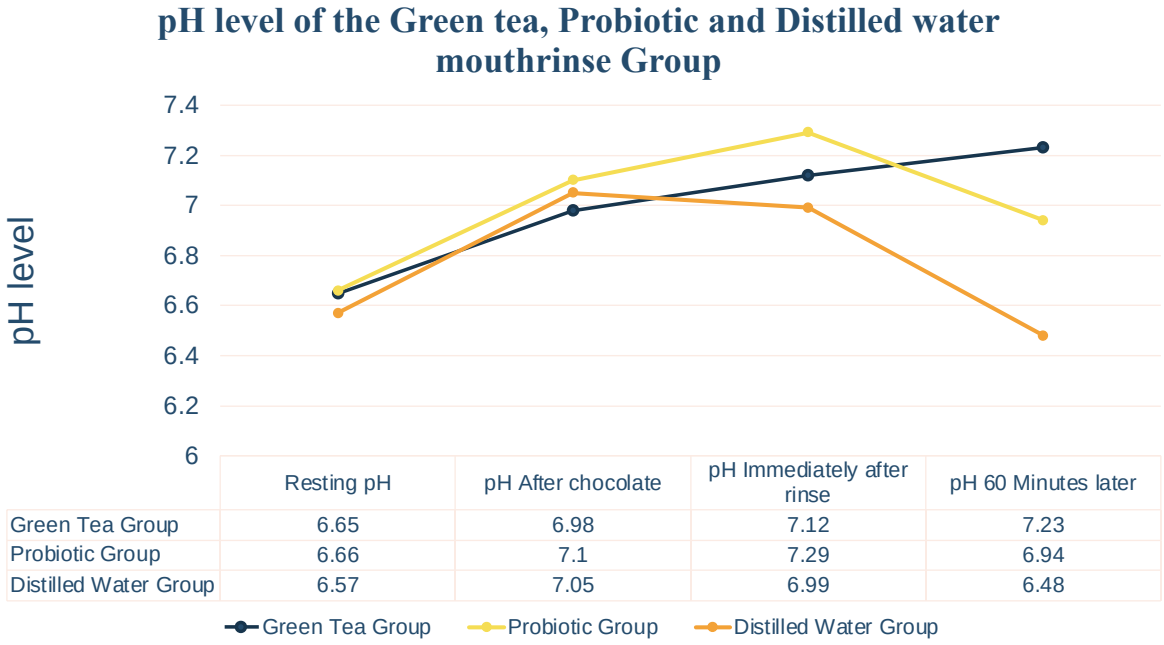
Results

Table 1 shows demographic details of the participants with no significant difference between age and sex in all the three groups.

	Green Tea Group n=15	Probiotic Group n=15	Distilled water Group n=15	Comparative Statistics	Significance
Age (Mean ± SD)	8.00±2.3	7.46±2.06	7.66±1.95	F= 0.242*	p= 0.786
Gender [n (%)]					
Male	9 (60)	10 (66.7)	8 (53.3)	χ^2= 0.556**	p=0.712
Female	6 (40)	5 (33.3)	7 (46.7)		

Table 1: Demographic characteristics of the Green tea, Probiotic and Distilled water mouth rinse Group. *One-way ANOVA **Fisher's Exact Test

Graph 1 shows the comparison of mean pH scores for green tea, which revealed that the pH of saliva was increased in the children after rinsing with green tea from 6.65 to 7.23 and was highly statistically significant ($p<0.001$). Similarly, when pre and post mean pH was compared in the probiotic group pH increased from 6.66 to 6.94 which were also highly statistically significant. In the distilled water group, the pH decreased from 6.57 to 6.48



ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

Groups	N	Resting pH	pH After chocolate	pH Immediately after rinse	pH 60 Minutes later	p
Green Tea Group	15	6.65±0.39	6.98±0.27	7.12±0.30	7.23±0.29	**0.000***
Probiotic Group	15	6.66±0.291	7.10±0.10	7.29±0.16	6.94±0.35	**0.000***
Distilled Water Group	15	6.57±0.34	7.05±0.11	6.99±0.26	6.48±0.57	**0.000***
p		0.735	0.205	**0.009***	**0.000***	

Table 2: Mean and standard deviation of pH level of the Green tea, Probiotic and Distilled water mouth rinse Group at Resting, after chocolate consumption, immediately after rinse and 60 minutes after rinse time interval using ANOVA and Tukey's post hoc analysis

* **p<0.05**

Discussion

This study sought to determine the impact of green tea and probiotic mouthwash on children's salivary pH. One of the most important markers of the carious process is salivary pH.[4] Saliva's pH ranges from 6.5 to 7.0 and is 6.8 on average when at rest. According to research, the selecting factor for the onset and advancement of caries is pH rather than sugar. Low salivary pH encourages the growth of aciduric bacteria, which then makes room for the spread of acidogenic bacteria and makes the environment unfavourable for the protective oral bacteria. This makes it possible for the environmental balance to change in favour of cariogenic bacteria, thus lowering the pH of the saliva. The plaque biofilms can be changed, the existing lesions can be remineralized, and perhaps the disease can be completely avoided by managing pH.[1]

The average salivary pH response to chocolate in this study displays an initial fast spike, followed by a fall in pH, hitting an after 5 minutes, and gradually reverting to resting levels after about 60 minutes. When unfilled chocolate was examined in a another study by Hegde M et al., milk chocolate exhibited the greatest pH drop. The positive characteristics of the cocoa mass, which are present in all chocolates, may be cancelled out by the milk when milk is added to the chocolate to form milk chocolates, and this could make the milk chocolate more cariogenic than the other chocolates.[10]

According to investigations by Tayab et al. and Pachori et al., it was observed that the average salivary pH responds to chocolate with an initial rapid spike and a subsequent pH decline.[11,12] . In the study conducted by Srinidhi et al., the increase

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

in salivary pH following rinse with black and green tea was also noted, with the pH increase being greater with green tea consumption compared to black tea.[13] When compared to the baseline, it was found that using a green tea mouthrinse maintained salivary alkalinity with statistically significant results (P 0.000). Tea's fluoride levels, polyphenols, and catechin content have been demonstrated in studies to inhibit the formation of caries. Green tea contains catechins, which have a noticeable impact on the pH of saliva and tooth plaque. By tracking the glycolytic pH decline of the Streptococcus mutans culture, Xu et al. in their thorough study ascertained the influence of EGCG on acid generation by S. mutans. At subminimum inhibitory doses, EGCG considerably reduced the amount of acid produced by S. mutans cells.[14] According to a study by Hamilton-Miller, rinsing with green tea catechins for a suitable amount of time can stop acid production and keep pH levels within the normal range (7.2–7.4), which are not conducive to the growth of Streptococcus mutans. He also claimed that green tea has anticariogenic and antibacterial properties.[15] Plaque pH levels were measured by Hirasawa et al. before and after being rinsed with 2% green tea for 5 min. They discovered that the pH values were much higher after treatment with catechins. According to the results of the current investigation, the probiotic mouthrinse group's salivary pH increased statistically substantially from baseline.[16]

When probiotic powder containing a combination of bacteria was used as mouthrinse for 14 days, mutans streptococcus count in saliva decreased statistically significantly, according to a study by Jindal et al. [17] Similarly, in the current study, saliva alkalinity was maintained compared to baseline when probiotic powder containing a combination of bacteria was used as mouthrinse. An efficient instructional tool, GC pH strips were utilised in the current study to test the salivary pH utilising a patient-specific approach.

The limitations of the current study was the smaller sample size. Long-term clinical trials are also necessary to assess the effectiveness of probiotic and green tea mouthwash on salivary pH in addition to microbiological assessment.

Conclusion:

Given the trial's constraints and findings, we can say that both green tea and probiotic mouthwashes were very significant in their ability to reverse the salivary pH following a chocolate challenge and maintain it for 60 minutes.

References:

1. Hurlbutt M, Novy B, Young D. Dental caries: A pH-mediated disease. CA Dent Hyg J 2010;25:9-15.
2. Sivapathasundaram B, Raghu AR. Dental Caries. In: Rajendran R, Sivapathasundaram B, editor. Shafer’s Textbook of Oral Pathology, 6th ed. New Delhi: Elsevier Publishers; 2009. p. 409-73.
3. Young WG. The oral medicine of tooth wear. Aust Dent J 2001;46:236-50.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

4. Shetty C, Hegde MN, Devadiga D. Correlation between dental caries with salivary flow, pH, and buffering capacity in adult South Indian population: An *in-vivo* study. Int J Res Ayurveda Pharm 2013;4:219-23.
5. M Hegde A, Shetty R, Sequeira AR. The acidogenicity of various chocolates available in Indian market: a comparative study. *Int J Clin Pediatr Dent*. 2009;2(2):20-24. doi:10.5005/jp-journals-10005-1025
6. Thakkar PK, Imranulla M, Naveen Kumar PG, Prashant GM, Sakeenabi B, Sushanth VH. Effect of probiotic mouthrinse on dental plaque accumulation: A randomized controlled trial. Dent Med Res 2013;1:7-12.
7. Neturi RS, Srinivas R, Simha VB, Sree SY, Shekhar CT, Kumar SP. Effects of green tea on *Streptococcus mutans* counts – A randomised control trail. J Clin Diagn Res 2014;8:128-30.
8. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694:72-7.
8. Jain I, Jain P. Comparative evaluation of antimicrobial efficacy of three different formulations of mouth rinses with multi-herbal mouth rinse. J Indian Soc Pedod Prev Dent 2016;34:315-23.
9. Coulter C, Walsh LJ. Saliva testing: Good practice, good sense. Stepwise 2006;1:32-5.
10. M Hegde A, Shetty R, Sequeira AR. The acidogenicity of various chocolates available in Indian market: a comparative study. *Int J Clin Pediatr Dent*. 2009;2(2):20-24. doi:10.5005/jp-journals-10005-1025
11. Tayab T, Rai K, Kumari V, Thomas E. Effect of chewing paneer and cheese on salivary acidogenicity: A comparative study. Int J Clin Pediatr Dent 2012;5:20-4.
12. Pachori A, Kambalimath H, Maran S, Niranjan B, Bhambhani G, Malhotra G. Evaluation of Changes in Salivary pH after Intake of Different Eatables and Beverages in Children at Different Time Intervals. Int J Clin Pediatr Dent 2018;11(3):177-182.
13. Srinidhi PB, Basha S, Naveen Kumar P, Prashant GM, Sushanth VH, Imranulla M. Effect of two different commercially available tea products on salivary pH: A randomized double blinded concurrent parallel study. Dent Med Res 2014;2:39-42.
14. Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. Antimicrob Agents Chemother 2011;55:1229-36.
15. Hamilton-Miller JM. Anti-cariogenic properties of tea (*Camellia sinensis*). J Med Microbiol 2001;50:299-302.
16. Hirasawa M, Takada K, Otake S. Inhibition of acid production in dental plaque bacteria by green tea catechins. Caries Res 2006;40:265-70.
17. Jindal G, Pandey RK, Agarwal J, Singh M. A comparative evaluation of probiotics on salivary *Mutans streptococci* counts in Indian children. Eur Arch Paediatr Dent 2011;12:211-5.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE03, 2021

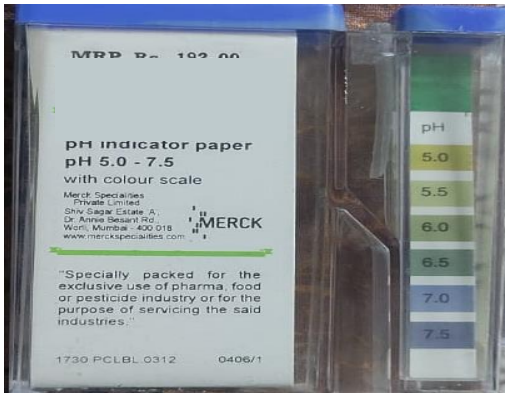


Figure 1 Merck pH strips

Figure 2 Probiotic and green tea

Figure 3 10 ml measuring cup and chocolate

Figure 4: pH papers

Figure 6 Measurement of pH

Figure 5 During mouth rinse