

An *In vivo* Comparison of Plaque pH Changes in Children Aged 8–12 Years after Consumption of Milk and Green Tea with Sugar

Nidhi Talreja¹, Shashikiran Nandihalli Devendrappa², Shilpy S. Singla³, Nikita Agrawal¹, Shikha Mali¹

¹Department of Paedodontics and Preventive Dentistry, Peoples College of Dental Sciences, ³Department of Paedodontics and Preventive Dentistry, Rishiraj College of Dental Sciences, Bhopal, Madhya Pradesh, ²Department of Paedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, India

Abstract

Aims: A study was conducted with an intent to compare the changes in plaque pH after rinsing with sucrose (arch criminal), milk with sugar, and green tea with sugar. **Materials and Methods:** A randomized, open-label, single exposure study was conducted in 30 children (aged 8–12 years). Plaque pH was measured after rinsing with sucrose (control group; $n = 10$), milk with sugar ($n = 10$), and 2% green tea with sugar ($n = 10$) at an interval of 5, 10, 20, and 30 min (short-term clinical trial). **Results:** Difference in mean plaque pH values between the groups and at different time intervals was statistically analyzed using one-way analysis of variance (ANOVA) and repeated measures ANOVA. The final plaque pH results revealed that in spite of adding sugar, green tea showed statistically significant difference when compared to comparators. **Conclusion:** The present study recommends the use of green tea with sugar in children as it is a potent caries preventive measure which prevents drop in plaque pH.

Keywords: Dental caries, green tea, plaque pH

INTRODUCTION

In the recent years, changing lifestyle and dietary factors have led to a tremendous increase in the consumption of sugar-containing beverages among children. According to the American Dental Association 2008, children consuming sweetened soda and fruit juices were almost twice at risk to develop cavities when compared with milk consumption. It is a known fact that unfavorable dietary patterns and frequent intake of sugar along with inadequate oral hygiene are important etiological combinations for dental caries.^[1]

However, encouraging the use of certain beneficial beverages such as milk and green tea can be a promising aid in preventing dental caries. Milk, universally endorsed for growing children on account of its variety of health benefits, is considered as less cariogenic due to the presence of casein.^[2] Furthermore, added to the anticariogenic family, we have green tea that has recently gained interest as a plant-derived stimulant beverage because of its antibacterial and anticariogenic properties. Catechins and polyphenols in green tea have a significant association with

plaque pH by maintaining it within normal range even after the consumption of acidic products.^[3]

The four major catechins present in green tea are epigallocatechin-3-gallate (EGCG) (59% approximately), epigallocatechin (19% approximately), epicatechin-3-gallate (13.6% approximately), and epicatechin (6.4% approximately). Green tea also contains gallic acid and other phenolic acids such as chlorogenic acid and caffeic acid, and flavonols such as kaempferol, myricetin, and quercetin.^[3] Apart from their polyphenol content, it is a natural source of fluoride and an effective vehicle for fluoride delivery to the oral cavity.^[4] All these contents thus favor the strong recommendation for this product as an effective anticariogenic agent in children. The only task would, however, be to make it palatable so that its consumption can be increased.

Address for correspondence: Dr. Nidhi Talreja, A1703, Jasmine Towers, SS2/72, Petaling Jaya, Selangor, Malaysia. E-mail: nidhitalreja@gmail.com

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How to cite this article: Talreja N, Devendrappa SN, Singla SS, Agrawal N, Mali S. An *in vivo* comparison of plaque pH changes in children aged 8-12 years after consumption of milk and green tea with sugar. *J Int Oral Health* 2018;10:10-5.

Access this article online

Quick Response Code:



Website:
www.jioh.org

DOI:
10.4103/jioh.jioh_195_17

Thus, the present study intended at evaluating the anticariogenic effects of green tea and milk with sugar in the oral cavity by measuring the plaque pH changes in children aged 8–12 years and comparing them with sucrose.

MATERIALS AND METHODS

An open-label, randomized, single exposure study was conducted over a period of 1 week in 30 children (aged 8–12 years) who were selected by simple random sampling. Being a proof of concept study, the sample size was decided based on the availability of participants and prevalence of plaque in children of visiting school. The participants were selected from the same school after obtaining the required Institutional Ethical Committee approval (PCDS/Acad/2963A).

Oral cavity examination was carried out, and participants were selected as per the following inclusion and exclusion criteria:

Inclusion criteria

- Healthy cooperative participants
- No history of oral prophylaxis in the past 3 months
- No orthodontic appliances.

Exclusion criteria

- History of fluoride or mouthwash in the past 48 h
- Participants who ate or drank in the past 2 h
- History of antibiotic therapy in the past 3 months.

Carious lesions if present were taken into consideration for appropriate treatment after the study. Antibiotics, fluoride, and mouthwash will suppress the number of bacteria in the plaque and may lead to false-positive results; hence, participants with a history of any kind of antibiotic therapy during the past 3 weeks, topical fluoride and topical mouthwash application in the past 48 h, and participants who ate or drank in the past 2 h before the study were excluded from the study.^[5]

Procedure

Eligible participants and their parents were given an informational summary to read, and the study was explained verbally. A signed informed consent form was obtained from the parents. All the demographic data and medical history (especially diabetes and allergy) were taken. Emphasis was given on history of diabetes as sugar is being used in the study to make the green tea palatable for the children, and also, allergic reactions to any of the components of green tea were taken into consideration while selecting the participants.

Appointments were arranged, and the approved participants were asked to refrain from all oral hygiene measures for 48 h so that sufficient plaque accumulation could occur. This time interval is in accordance with a study done by Telgi *et al.*^[6] and is considered acceptable for plaque accumulation.

A prerinse baseline pH was measured for all the 30 selected children.

They were then randomly divided into three groups of 10 each. Group 1: 10% sucrose already prepared (Qualigens fine

chemicals, Mumbai, Maharashtra, India), Group 2: milk with sugar, and Group 3: 2% green tea with sugar.

Preparation of solutions

Two percent green tea (Organic India, Lucknow, Uttar Pradesh, India) was prepared by adding 2 g in 100 ml of deionized water (Ultrapure, Organo Biotech Laboratories, New Delhi, India) and brewed for a constant period of 5 min.^[7] An equal quantity of sugar, i.e., 1 teaspoon (approximately 5 g) was added to both the test samples, green tea and milk (bovine packed milk, pH - 6.5–6.7 and temperature - 7°C).

Students were then made to rinse their mouth with the respective solution as per the allotted intervention for 2 min, and plaque samples were collected [Figure 1] after 5, 10, 20, and 30 min.

One gram per sample was collected from lingual surface of permanent maxillary right first molar and palatal surface of permanent mandibular left first molar, permanent mandibular left first premolar, permanent mandibular right first molar, and permanent mandibular right first premolar using four occlusally directed strokes with the help of sterile curettes or probes and was then stored in sterile polypropylene tubes. They were then transferred to laboratory for further biochemical analysis.

After the collection of plaque samples, i.e., after 30 min, students were made to rinse vigorously with distilled water to prevent pooling of the test samples in their mouth.

Plaque pH testing

The procedure was carried out in Clinical Science and Research Development Laboratory. Calibration of pH meter (Eutech Digital pH meter, Switzerland) was done using buffer solutions with pH 4 and 7 (Sensorex, USA), prepared using the buffer capsules, every day before the commencement of experiment. pH was separately evaluated for each time interval, and 1 g of plaque was dissolved in 1–2 ml of distilled water^[3] [Figure 2]. The electrodes were cleaned and blotted dry, and tip of the electrode was placed in the plaque suspension [Figure 3]. It was held in position until the readings on the display unit stabilized. The electrode was rinsed with distilled deionized water after each reading to prevent cross-contamination.



Figure 1: Collection of plaque samples

RESULTS

Difference in mean plaque pH values between the groups and at different time intervals was statistically analyzed using one-way analysis of variance (ANOVA) and repeated measures ANOVA. Data were analyzed using the IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp.).

Table 1 shows the mean and standard deviation (SD) of plaque pH in experimental groups at different time intervals in between the three groups. There was no significant difference between the groups for prerinsing values. At 5, 10, 20, and 30 min, one-way ANOVA showed a significant difference in plaque pH between the three experimental groups. When least significant difference (LSD) *post hoc* test was applied, the lowest pH was observed in 10% sucrose group and the highest in 2% green tea group at all time intervals (except prerinsing).

Tables 2–4 depict the comparison of plaque pH in 10% sucrose group, milk group, and green tea group, respectively, at different time intervals using repeated measures one-way ANOVA. Results showed a significant difference in plaque pH in 10% sucrose group at different time intervals. When LSD *post hoc* test was applied for pairwise comparison, it showed that at prerinsing, plaque pH was significantly higher than at 5, 10, and 20 min. Differences in plaque pH at other time intervals were not significant.

For Group 2, repeated measures one-way ANOVA showed a significant difference in plaque pH in milk group at different time intervals. When LSD *post hoc* test was applied for pairwise comparison, it showed that at 5 and 20 min, plaque pH was significantly higher than pH at prerinsing and 10 min. At 30 min, it was significantly higher than plaque pH at prerinsing, 10 min, and 20 min. Differences in plaque pH at other time intervals were not significant. For Group 3, significant difference in plaque pH in 2% green tea group at different time intervals was seen. When LSD *post hoc* test was applied for pairwise comparison, it showed that 30-min plaque pH was significantly higher than other time intervals. The lowest pH was observed at prerinsing.

DISCUSSION

Dental caries can be averted by eliminating the etiologic factors and incorporation of milk and herbal compounds such as green tea in one's diet. This will not only aid in maintaining the plaque pH but also decrease acidogenicity and thus reducing the risk of demineralization. As already described by Stephan in his classic studies in the early 1940s, dental plaque exposed to sucrose leads to acid production, causing a rapid drop in pH leading to the initiation of caries.^[8] Hence, acid production is an important trait of cariogenic bacteria and its assessment by measuring plaque pH can be a useful indicator of caries risk assessment.^[8] Addition of sugar to both the proven

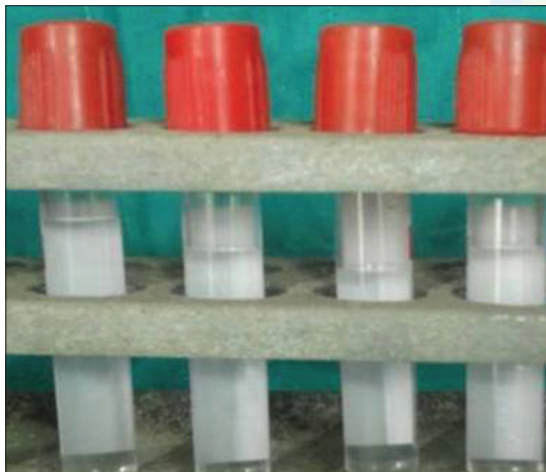


Figure 2: Dilution of plaque samples



Figure 3: Digital pH meter

Table 1: Mean and Standard Deviation (SD) of plaque pH in experimental groups at different time intervals

Experimental groups	n	Pre-rinsing (mean±SD)	5 min (mean±SD)	10 min (Mean±SD)	20 min (mean±SD)	30 min (mean±SD)
10% sucrose group (1)	10	6.17±0.20	6.01±0.16	5.98±0.18	6.05±0.23	6.16±0.18
Milk group (2)	10	6.21±0.24	6.45±0.10	6.37±0.08	6.46±0.05	6.50±0.04
2% Green tea group (3)	10	6.14±0.12	6.69±0.08	6.78±0.11	6.83±0.11	6.93±0.09
One way ANOVA		0.337* P=0.717 (>0.05)	83.488* P=0.0001 (<0.001)	92.381* P=0.0001 (<0.001)	69.901* P=0.0001 (<0.001)	106.717* P=0.0001 (<0.001)
LSD <i>post hoc</i> test (significant results)		Not Applicable	2>1 3>1,2	2>1 3>1,2	2>1 3>1,2	2>1 3>1,2

For LSD Post Hoc, 1=Group 1, 2=Group 2, 3=Group 3

Table 2: Comparison of plaque pH in 10% Sucrose group at different time intervals using Repeated Measures one way ANOVA

F	P	LSD post hoc test* (significant results)
3.025	0.030 (<0.05)	1>2,3,4

*For LSD post hoc 1=Pre-rinsing, 2=5 Min, 3=10 Min, 4=20 min, 5=30 min

Table 3: Comparison of plaque pH in Milk group at different time intervals using Repeated Measures one way ANOVA

F	P	LSD post hoc test* (significant results)
10.294	0.005 (<0.01)	2,4>1,3 5>1,3,4

*For LSD post hoc 1=Pre-rinsing, 2=5 Min, 3=10 Min, 4=20 min, 5=30 min

Table 4: Comparison of plaque pH in 2% green tea group at different time intervals using repeated measures one way ANOVA

F	P	LSD post hoc test* (significant results)
140.785	0.0001 (<0.001)	2>1 3>1,2 4>1,2,3 5>1,2,3,4

*For LSD post hoc 1=Pre-rinsing, 2=5 Min, 3=10 Min, 4=20 min, 5=30 min

anticariogenic agents, i.e., milk and green tea, was essential to make the drinks palatable for children. Thus, the present study intended to compare plaque pH change after consumption of green tea and milk with sugar which has not been addressed in literature till now, with an objective to evaluate the effect of sweetened milk and green tea on plaque pH knowing the fact that it can be easily ingested by children with added benefits of anticariogenicity of the two agents.

The present study includes school-going children of 8–12 years as developing permanent dentition and changing dietary patterns make them more susceptible to develop caries.^[9,10]

Several studies have indicate that in children and adolescents, sugar-sweetened beverage consumption has increased and milk consumption has decreased over the past 20 years.^[11,12]

Milk is considered essential for this age group as it provides an important source of dietary calcium, and decline in the consumption of milk, especially by girls, at a time, when bone mineral deposition is to occur, may predispose to eventual osteoporosis which is a major concern.^[13] This substitution of milk by sugar-containing beverages may be attributed to the conveniently available various vending machines at school;^[14] furthermore, many schools have “tie-ups” with their beverage suppliers, and they are greatly benefitted in proportion to beverage sales.

Hence, essential steps are necessary to reverse these advancing trends. Studies have demonstrated that although sugar

consumption can contribute to tooth decay, sweetened milk in the form of flavored milk is considered as a good alternative due to the presence of casein, calcium, phosphorus, proteins, and other components. Furthermore, sweetened milk being liquid, it is comparatively less cariogenic than raisins and candies that adhere to the tooth surface.^[15] A briefing paper on flavored milk, milk products and dental caries concluded that cariogenicity of flavored milk is negligible to low and can be consumed in moderation. The American Academy of Pediatrics in a policy statement on soft drinks in schools, recommends restriction on the sale of soft drinks in schools^[16] and in another report on optimizing children’s and adolescents’ bone health and calcium intakes,^[17] encourages consumption of nutritious beverages including low-fat or fat-free flavored milk with modest amounts of added sweeteners. Furthermore, in a systematic review, Harris *et al.* 2004^[18] concluded that consumption of sugars and sugary foods did not appear to be associated with caries for children who brushed their teeth twice a day or more. Thus, keeping in mind, the enormous benefits that could be achieved by adding a pinch of sugar and sweetened milk were chosen for mouth rinsing to promote its increased use in children.

In the present study, another natural product used was green tea, and it is said to considerably prevent drop in plaque pH on oral rinsing as explained by Awadalla *et al.*^[3] Green tea being a very popular and widely consumed beverage^[3,19] showed antibacterial properties chiefly because of catechins. Lee *et al.*^[20] revealed that the catechins were retained in saliva for 60 min and preserved plaque pH toward neutrality.^[3] The catechins possess antiplaque and antibacterial properties by interacting with barrier function of microorganism and depletion of this group, thus contributing to caries prevention.^[3] Furthermore, Otake *et al.*^[21] studied the anticaries effect of green tea polyphenols *in vivo*, and they reported that polyphenolic compounds present in green tea possess high-inhibitory effect against *Streptococcus mutans* bacteria growth and acid produced from it and that was the main cause of its anticaries effect. Moreover, 5-min brewing produces the best flavor with least extraction of unpleasant tasting tannin.^[6] The additional advantage was fluoride content of milk which was found to be in the range of 0.01–0.051 mg/kg and that of tea was 0.005–371 mg/kg, which is said to increase with the brewing time.^[22]

Moreover, Zhang and Kashket^[23] concluded that green tea extracts inhibit human salivary amylase and may decrease the cariogenic potential of starch-containing foods such as crackers and cakes because the propensity of this form of food to serve as slow-release sources of fermentable carbohydrate may be reduced which greatly contributed to the idea of adding sugar to green tea for making it palatable and harnessing its beneficial effects for children as well.

In the present study, 10% sucrose was used for 2 min as a control group.^[24] This was done so as to standardize the baseline for the study group. Sgan-Cohen *et al.* reported that rinsing mouth for 1 min with 10% sucrose after fasting

overnight exhibits a plaque pH of 5.5 or below.^[25] In the present study, a prerinsing plaque pH was significantly higher than the pH after sucrose rinse at 5, 10, and 20 min.

Plaque pH was checked after 5, 10, 20, and 30 min interval which was in accordance to Mortazavi and Noin.^[24] This time interval appeared to be correct as it reduced the ability of saliva to buffer the pH as the samples were being monitored. Thus, this technique involved a diminished salivary effect, which was similar to that seen if at all the sweetened products are made to pool around the tooth surface which was not the case in the present study as the selected children were made to rinse with distilled water post-rinsing with the test samples.

To prevent such drop in pH, certain natural beverages were investigated. Reynolds^[26] postulated that increase in plaque pH occurred when milk peptides and amino acids were formed from the hydrolysis of casein.^[26] According to Aimutis WR,^[27] milk-derived bioactive peptides had shown inhibition of cariogenic bacteria and tooth enamel demineralization with subsequent enamel remineralization.^[27] In the present study, milk showed significantly higher pH than 10% sucrose which is in accordance with Koparal *et al.*^[28] and Bowen and Lawrence.^[29] However, sugar was not added in these studies. This rise could be attributed to the alkaline environment produced by casein which counteracts the acid produced from fermentation of lactose and the added sugars.

In the present study, at all the time intervals, the highest pH was seen with 2% green tea. These results were in consequent with another study which reported that only 5-min exposure of cariogenic bacteria to green tea solution resulted in >50% decrease in colony-forming unit of *S. mutans*.^[30] These beneficial effects can be mainly attributed to catechin content, primarily EGCG, and the polyphenol content, mainly the flavonoids which constitute 30% of the fresh leaf dry weight. Significant difference in pH was seen in spite of adding sugar to green tea which thus promotes increased acceptance in children who will be greatly benefited by this natural product.

Thus, the present study demonstrated that green tea maintains plaque pH under acidic environment and aids in preventing dental caries with maximum effect seen at 30-min interval. Green tea can be used as a substitute for carbohydrate-laden desserts and snacks and thus may help reduce the incidence of dental caries. However, for a precise evaluation, a study with larger sample size should be conducted.

Limitations of the current study are that it is a proof of concept study, so a study with larger sample size and longer duration is required to confirm the findings from this study. Furthermore, children were exposed to benefits of green tea, but addition of sugar (though in small quantity) might have masked further potential benefit of green tea. An alternate solution could be to find another way of exposure to green tea for children.

Some of the controversies that still exist are whether children should be exposed to green tea, and if yes, then duration of

exposure is another issue to address as little has been known of potential benefits and harm of green tea over long period of time. There are many studies stating the adverse effects of green tea for children, which is mainly due to the caffeine content which may cause insomnia, inability to concentrate, irritability, or hyperactivity.^[31] However, in our study, children can be considered absolutely safe as green tea is used only for its topical benefits and thus all the systemic side effects are avoided. Furthermore, the dilution at which green tea is being used in this study seems difficult to cause any harm to children.

CONCLUSION

The results of the present study proved that local application (oral rinsing) with green tea solution for short time significantly inhibits decrease in plaque pH. Hence, regular oral application of green tea solution in the form of mouthwashes or its addition to dentifrices might be a cost-effective caries preventive measure, especially in developing countries. Besides this, green tea cakes muffins, pastries, and ice-creams are available nowadays proving to be a treat for taste buds of children.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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