ORIGINAL RESEARCH

Comparative Evaluation of Changes in Salivary pH and *Streptococcus mutans* Count in Saliva by Natural Sugar Substitutes: An *In Vivo* Study

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Abstract

Aim: The purpose of this study was to compare the effect of natural sugar substitutes—stevia, jaggery, and honey on salivary pH and *Streptococcus mutans (S. mutans)* count in saliva.

Materials and methods: Children aged between 7 and 12 years with no active carious lesion were selected. A total of 80 subjects were randomly selected and divided into three experimental groups and one control group, with 20 subjects in each group. Prior to rinsing with the respective solutions, the baseline pH and *S. mutans* count were evaluated for each individual. All solutions were freshly prepared whenever required. After rinsing with the respective solutions, saliva samples were collected to evaluate pH and *S. mutans* count. The pH was analyzed at different time intervals, that is, 0 (immediately after rinsing), 15, and 30 minutes. The *S. mutans* count was analyzed after 30 minutes of rinsing with the respective solutions.

Results: The results were tabulated and statistically analyzed using one-way analysis of variance (ANOVA) and *post hoc* tests. The results depicted that group I (stevia) showed a maximum increase in salivary pH, followed by group III (honey) and group III (jaggery) at different time intervals (0, 15, and 30 minutes). The maximum number of subjects who had shown a decrease in *S. mutans* count were from group III (honey), followed by group I (stevia), and group II (jaggery) after 30 minutes of rinsing with the respective solutions when compared to baseline *S. mutans* count.

Clinical significance: It is a universally known fact that dental caries is a multifactorial disease process, one of the key factors of which is the consumption of sugar. Of all the fermentable carbohydrates, sucrose is considered the archcriminal in the carious process. Hence, this advocates the need for developing suitable sugar substitutes that help in controlling dental caries. An ideal sugar substitute should not only minimize the risk of dental caries but also should have nutritional benefits.

Conclusion: Natural sugar substitutes (stevia, jaggery, and honey) have the ability to reduce caries risk in children.

Keywords: Honey, Jaggery, Natural sugar substitutes, Salivary pH, Stevia, *Streptococcus mutans.*

International Journal of Clinical Pediatric Dentistry (2023): 10.5005/jp-journals-10005-2672

Introduction

Dental caries is the most prevalent oral disease among children. It is a chronic and multifactorial disease in which there is an interaction among cariogenic microflora, a diet rich in fermentable carbohydrates, and host factors over time.[1] According to the National Health and Nutrition Examination Survey, the prevalence of dental caries in children aged 2–11 years had increased from 40% (1988–1994) to 42% (1999–2004). Currently, this prevalence of dental caries has increased to 49%, based on the Global Oral Health Data Bank.[2] Dietary studies have proved that the incidence and prevalence of dental caries can be moderated by reducing the frequency and type of sugar consumption.[3]

Sucrose has been known as the "archcriminal" as it is considered highly cariogenic. It has the unique ability to support the synthesis of extracellular (water-soluble and water-insoluble) glucans by *Streptococcus mutans (S. mutans)*, enhancing its accumulation in the plaque. It increases the porosity of plaque, permitting deeper penetration of dietary sugars and greater acid production adjacent to the tooth surface.[4]

Some of the key issues to be considered in deciding cariogenic, cariostatic, and anticariogenic properties of diet are its form, frequency, retention of food, and capacity of food to stimulate salivary secretion. If cariogenic food is retained for a longer period of time in the oral cavity, it increases the duration to which teeth are exposed to low acidic pH. Hence, prolonged exposure to sugar increases the caries risk.[3] According to the Vipeholm Study, the frequency and physical form of eating sugars are more critical factors compared to the total consumption of sugars in the etiology of dental caries. This results in increased retention time and prolonged pH fall.[4]

With the increased knowledge of the concept of dental caries, it has been understood that the caries process is a continuum resulting from many cycles of demineralization and remineralization. Due to its dynamic developmental characteristics, the carious lesion can be arrested and even repaired in its early

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How to cite this article: Sharma W, Bhola M, Bajaj N, *et al.* Comparative Evaluation of Changes in Salivary pH and *Streptococcus mutans* Count in Saliva by Natural Sugar Substitutes: An *In Vivo* Study. Int J Clin Pediatr Dent 2023;16(5):728–733.

Source of support: Nil

Conflict of interest: None

stages without surgical intervention by increasing the net mineral gain during the cycles of demineralization and remineralization. This can be achieved by modifying one of the etiological factors, diet, in such a way that it does not lead to demineralization.[5]

Recently, sugar substitutes have been preferred due to their lower calorie content. Sugar substitutes tend to be sweet but are not metabolized by cariogenic organisms and, therefore, cannot lower the biofilm pH on the tooth surface. Research for alternatives to sucrose has led to the development of artificial sweeteners, many of which are considered less cariogenic, such as aspartame, saccharin, cyclamate, xylitol, and mannitol.[6] Advancing research in sugar substitutes has concentrated on the identification and evaluation of natural sweeteners that are less cariogenic. Recently, a sharp upward trend has been seen in the usage of natural products in dentistry. Commonly preferred natural sweeteners are licorice, palm sugar, stevia, honey, and jaggery.

Stevia rebaudiana bertoni is stevia's organic name. It is called Paraguay's sweet herb because it is indigenous to Paraguay and Brazil. Main steviol glycosides are stevioside (110–220 times sweeter than sugar) and rebaudioside A (180–400 times sweeter than sugar), which are its major component. Giacaman et al. revealed that the decrease in the production of extra and intracellular polysaccharides leads to a decrease in the hydrophobicity of the surface and the adhesion of bacteria to plaque.[7] Das et al. considered extracts from stevia not to be cariogenic. None of these compounds demonstrated the potential to increase the risk of caries development.[8]

Jaggery is a natural, traditional, and unrefined noncentrifugal sugar made by concentrating the extracted sugarcane juice, which is consumed in Asia, Africa, Latin America, and the Caribbean. Out of the total world production, >70% of it is produced in India. It also has higher medicinal and nutritional values and is easily available to rural people. It consists of long chains of sucrose that appear to lag the digestion process and release energy gradually rather than impromptu. Recent research suggested the use of jaggery as a sweetener due to the presence of various phenolic components, which indicates higher bioactivity, that is, cytoprotective and antioxidant activity.[9] Takara et al. found the two phenolic bioactive compounds from sugar cane molasses (dehydrodiconiferyl alcohol-90-O-b-D-glucopyranoside and isoorientin-7,30-O-dimethyl ether), which have inhibitory properties against the cariogenic bacteria *S. mutans* and *Streptococcus sobrinus* comparable to commercial antibacterial agents.[10]

Honey has been used in China, India, Greece, Rome, and many other nations since ancient Egyptian times. It contains 38% fructose, 31% glucose, 17% water, 10% other sugars, and a wide range of micronutrients (vitamins, amino acids, and minerals) and some enzymes like invertase, glucose oxidase, etc. It causes less demineralization when compared to glucose and fructose. As it is less cariogenic, it is used as a sweetener in toothpaste, gums, chocolates, etc. Active factors in honey's antimicrobial activity include the presence of hydrogen peroxide, flavonoids, high sugar concentration, and propolis.[11] By the action of the enzyme glucose oxidase, gluconic acid, and hydrogen peroxide are produced from glucose, which leads to a reduction in the bacterial colony.[12] High concentration of sugar produces a hypertonic condition, which causes plasmolysis of microbial cells, resulting in growth inhibition and death. Propolis is present in bee products, which possess antimicrobial activity against *S. mutans*. It can be used in the reduction of plaque accumulation and formation of polysaccharides.[13]

Most studies evaluated and compared the salivary pH and enamel demineralization depth of different sugar substitutes such as xylitol, sorbitol, aspartame, saccharin, sucralose, stevia, etc. The literature search revealed very few studies assessing and contrasting the changes in salivary pH and *S. mutans* counts after using various natural sugar substitutes such as stevia, honey, and jaggery. Earlier, research was carried out *in vitro* and *in vivo* only in adult patients. The aim of the present study was to evaluate and compare the changes in salivary pH and *S. mutans* count in saliva after mouth rinsing with various natural sugar substitutes.

Material and Methods

This study was conducted in the Department of Pediatric and Preventive Dentistry, Dasmesh Institute of Research and Dental Sciences, Faridkot. Ethical clearance was obtained from the Ethical Committee of the institution. Written informed consent was obtained from parents/guardians of the children who were selected for the study based upon inclusion and exclusion criteria.

Inclusion Criteria

- Children between the age group of 7 and 12 years.
- Children with no active carious lesions.
- Healthy children without any systemic diseases.
- Subjects with no history of any preventive dental treatment.

Exclusion Criteria

- Undergoing dental treatment.
- Those who were on any medication that would alter the salivary flow.
- Using any mouthwash for maintaining oral hygiene.
- Having any painful, debilitating oral condition.
- Presence of any systemic disease.
- Children who failed to comprehend the task.

A total of 80 children were randomly selected and divided into three experimental groups and one control group, depending on the solution used for mouth rinsing.

Group I: Stevia dissolved in distilled water ($n = 20$)
Group II: Jaggery dissolved in distilled water ($n = 20$)
Group III: Honey dissolved in distilled water ($n = 20$)
Group IV: Distilled water ($n = 20$)

Preparation of the Solution

- All the solutions were freshly prepared whenever required. The test solutions were prepared by using commercially available stevia, jaggery, and honey.
- A total of 10 gm of each sugar substitute was measured and then dissolved in 90 mL of distilled water according to the groups divided.

Method of Saliva Collection

After the selection of the subjects, they were randomly allocated to different groups. All subjects were given instructions to refrain from eating for 1 hour before the collection of saliva. Subjects were asked to accumulate the saliva within the floor of the mouth and then spit it out in the sterile container after 60 seconds. Collected saliva samples were evaluated for pH, and *S. mutans* count.

pH Evaluation

Prerinse saliva samples collected in the beaker were checked for pH using pH indicator strips (Indikrom Papers, Thermo Fisher Scientific Pvt. Ltd., India). The pH strip was dipped into saliva and was observed for 10 seconds for color change. The color change was compared with the reference chart provided in the kit, and readings were tabulated.

After determination of baseline pH, subjects were instructed to mouth rinse with the solutions of the respective groups. A total of 10 mL of freshly prepared respective solutions were given to the subjects. They were instructed to swish the respective solution in their mouth for 1 minute and then asked to spit it out in the sterile container. After rinsing with the respective solutions, salivary pH was evaluated at 0, 15, and 30 minutes.

Streptococcus mutans (S. mutans) Count Evaluation

Prerinse saliva samples collected in the sterile container were used for evaluation of *S. mutans* count using a commercially available kit (GC Saliva-Check Mutans, GC Corporation, Japan). It contains a test device, pipette, mixing container, reagent 1 (tris NaOH), and reagent 2 (tris citrate). This test device contains colloidal gold labeled anti-*S. mutans* monoclonal antibody. According to the manufacturer's instructions, a volume of 250 µL of saliva sample was added to the mixing container. One drop of reagent 1 was added to it, and the opening of the mixing container was folded and tapped 15 times for a period of 10 seconds. Then, four drops of reagent 2 were added and shaken for another 5 seconds till the color of the saliva sample changed to light green. A total of 0.3 mL of this mixture was dispensed into the sample window of the test device using a graduated pipette. This was left for 15 minutes to observe the lines on the control and test window.

A thick red line was observed in the control window, which indicated that the test device was working properly. The appearance of a red line in the test window indicated that the levels of *S. mutans* were equal to or above 5×10^5 CFU/mL saliva, and the results were considered positive (Fig 1). If no line was observed in the test window, the results were considered negative. The negative results depicted that the levels of *S. mutans* were below 5×10^5 CFU/mL saliva (Fig. 2).

After determining the baseline *S. mutans* count, the subjects were instructed to mouth rinse with the solutions of the respective groups. After 30 minutes of mouth rinsing with respective solutions, the *S. mutans* count was again measured using a commercially available kit (GC Saliva-Check Mutans, GC Corporation, Japan).

Results

The data for the present study was entered in Microsoft Excel 2013 and analyzed using the Statistical Package for the Social Sciences statistical software 19.0 version. The descriptive statistics included mean and standard deviation. The statistical analysis was done using one-way analysis of variance (ANOVA) and *post hoc* analysis.

Discussion

Despite a global decline in the prevalence of dental caries in recent years, it remains a significant, chronic disease of childhood that has a negative impact on health-related quality of life. Despite the use of various preventive measures, 10% consumption of sugar has been found to increase the incidence of caries, as per the World Health

Fig. 1: High caries risk 105 CFU/mL

Fig. 2: Low caries risk 105 CFU/mL

Organization 2015 document.[5] With a paradigm shift in approach to deal with this disease, an immense focus is now laid on preventive strategies along with the management of the carious lesions.

Frequent intake of sugar and sugar products favors the growth of cariogenic microorganisms, that is, *S. mutans*. Sucrose gets fermented into lactic acid and generates a low acidic pH environment, thereby causing demineralization of enamel. The pH and *S. mutans* are important parameters in the oral microbial ecology associated with dental caries. Hence, these are considered salivary biomarkers in assessing the caries risk in future in an individual. Considering diet as an important factor in the development of dental caries, sugar substitutes can be used as an effective and adjunctive preventive measure to control the development of dental caries.[3] Thus, sugar substitutes have been recommended as they cannot be metabolized by cariogenic microorganisms. Sugar substitutes can be classified as natural and artificial sugar substitutes or intense (noncaloric/nonnutritive) and bulk (caloric/nutritive) sweeteners. Artificial sweeteners are chemically synthesized and are used in various soft drinks, ice creams, confectioneries, etc. Some of the artificial substitutes are acesulfame K, alitame, neotame, aspartame, cyclamate, saccharin, and sucralose. Animal studies have proved that artificial sugar substitutes lead to various medical problems such as weight gain, brain tumors, bladder cancer, and many other health hazards. Advancing research has led to the development of safe and palatable natural sweeteners that are less cariogenic. The natural products have been successfully used in dentistry as antimicrobial plaque agents. These confer considerable antibacterial activity against various microorganisms, including microbes responsible for dental caries. Commonly preferred natural sweeteners are xylitol, sorbitol, licorice, palm sugar, stevia, honey, and jaggery.[5]

The research on natural sugar substitutes has focused on the cariogenic potential as well as nutritional aspects of these substitutes. The popularity of these sugar substitutes is growing rapidly throughout the world. Hence, advancing research in the area of natural sugar substitutes has concentrated on the identification and evaluation of substances that exhibit antimicrobial activity, too.

Table 1 and Figure 3 depict an intergroup comparison of mean salivary pH 0 (immediately), 15, and 30 minutes after rinsing with the respective solution. The mean salivary pH immediately (0 minutes) after rinsing with the respective solutions were 7.60 for group I (stevia), 7.55 for group II (jaggery), 7.05 for group III (honey), and 7.00 for group IV (distilled water). The highest mean value was seen in group I (stevia), followed by group II (jaggery) and group C (honey). This was in accordance with the study conducted by Brambilla et al. They concluded that after rinsing with stevia extracts (stevioside and rebaudioside A), there was an increase in the salivary pH from 0 to 45 minutes, which provided evidence that the stevia extracts could be considered as noncariogenic food.[14] In the study conducted by Mahtani et al., when participants rinsed with the commercially available stevia, results showed the least growth of *S. mutans* and the highest value of salivary pH from baseline to 20 minutes when compared to aspartame.[15] According to the study conducted by Ali et al., honey contains enzymes such as hydrogen peroxidase, which, when diluted, becomes a potent antibacterial rinse.[16] According to Razdan et al. and Jayadevan et al., honey showed less enamel demineralization when compared to other sweeteners.[5,17] A study conducted by Ahmadi-Motamayel et al.

Table 1: After rinsing with the various natural sugar substitutes, that is, groups I (stevia), II (jaggery), III (honey), and IV (distilled water), there was an increase in the salivary pH levels at 0 and 15 minutes in all the four groups; even after 30 minutes of rinsing, there had been no fall in salivary pH below the baseline salivary pH in any of the subjects; group I (stevia) showed maximum increase in salivary pH followed by group II (jaggery), and group III (honey) at different time intervals after rinsing with the respective solutions

		Postrinse salivary pH			
Groups	*Baseline pH*	*0 minute*	*15 minutes*	*30 minutes*	*p-value*
Group I (stevia)	6.85 + 0.48	7.60 + 0.66	7.75 + 0.62	7.45 + 0.50	0.001
Group II (jaggery)	7.15 + 0.65	7.55 + 0.80	7.30 + 0.64	7.05 + 0.50	0.11
Group III (honey)	7.05 + 0.67	7.05 + 0.74	7.25 + 0.83	7.05 + 0.67	0.78
Group IV (distilled water)	7.00 + 0.032	7.00 + 0.32	7.00 + 0.32	7.00 + 0.32	1.00

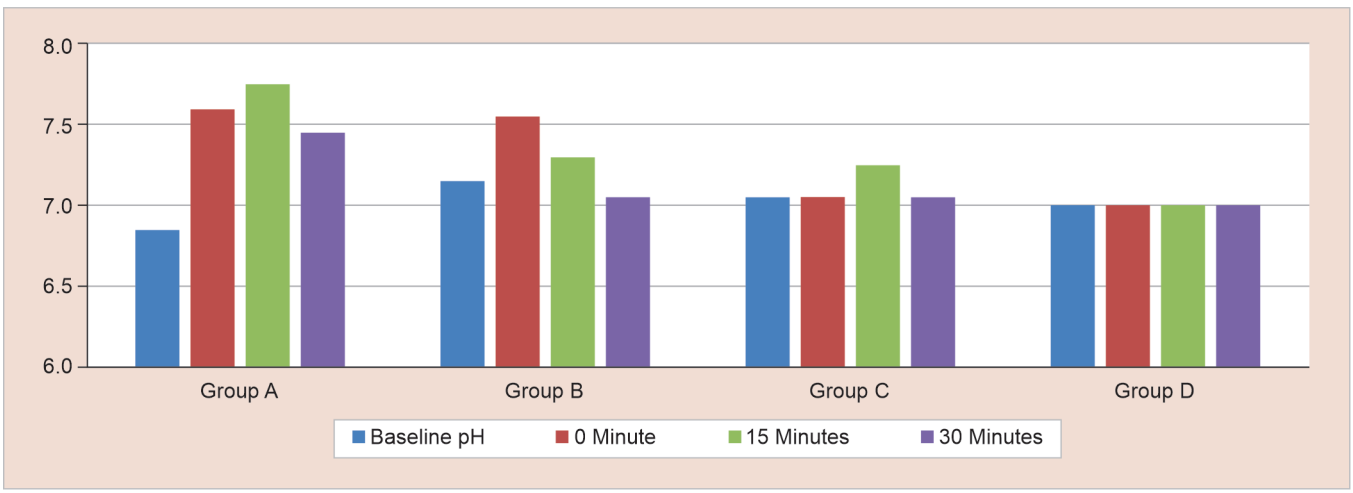

Fig. 3: Intergroup comparison of mean values of salivary pH of baseline and three postrinse samples in all four groups

Table 2: There was a statistically significant difference found in the number of subjects who showed a change in *S. mutans* count in all four groups; in group III (honey), the maximum number of subjects showed a decrease in the *S. mutans* count followed by groups I (stevia) and II (jaggery); there was no change in the *S. mutans* count after rinsing with distilled water (group IV)

	The number of subjects who showed					
Groups	*Increase in S. mutans count*	*Decrease in S. mutans count*	*No change in S. mutans count*	*Chi-squared value*	*p-value*	*Status*
Group I (stevia)	0	9	11	17.28	0.0006	Significant
Group II (jaggery)	0	9	11			
Group III (honey)	0	12	8			
Group IV (distilled water)	0	0	20			

showed that honey is less cariogenic when compared to glucose and fructose.[11] Molan found honey to be less cariogenic than sucrose, which suggested that honey can be used to control the initiation of dental caries.[18] Takara et al. found that the presence of phenolic bioactive compounds (dehydrodiconiferyl alcohol-90-O-b-D-glucopyranoside and isoorientin-7,30-O-dimethyl ether) present in the jaggery are might be responsible for less cariogenic activity. These compounds had inhibitory properties against S. *mutans* and *Streptococcus sobrinus*.[10] In the present study, distilled water was used as the control group because it is a nonelectrolyte and an inert substance. Before the distillation process, the pH of water is usually 5.58. After the distillation procedure, there was an increase in the pH of the water, that is, pH = 6.96, and it contains no impurities.[19,20]

Table 2 depicts the number of patients who showed a change in *S. mutans* count in all four groups, that is, group I (stevia), group II (jaggery), group III (honey), and group IV (distilled water). After rinsing with respective solutions, none of the subjects showed an increase in the *S. mutans* count. In group III (honey), the maximum number of subjects showed a decrease in the levels of *S. mutans,* followed by group I (stevia) and group II (jaggery). It might be due to the presence of various factors such as the osmotic effect, enzymatic glucose oxidation reaction, production of hydrogen peroxide, high osmotic pressure, a low pH, an acidic environment, and the presence of phenolic acids, lysozyme, flavonoids, phytochemicals, antioxidants, beeswax, nectar, pollen and propolis in the honey which are responsible for its antimicrobial action. Among all these factors, the presence of hydrogen peroxide in honey might be responsible for its potent antibacterial action.[4] Jayadevan et al. studied the effect of refined and natural sugar substitutes on *S. mutans* biofilm and found that xylitol (negative control) showed the least biofilm formation, followed by stevia, honey, and jaggery.[5] In the experiment conducted by Giacaman et al., it was noted that *Stevia rebaudiana* decreased the production of intracellular and extracellular polysaccharides.[7] This prevents the adhesion of bacteria to dental plaque. In group II (jaggery), the presence of phenolic compounds could be responsible for the antibacterial activity. The phenolic compounds are retained in jaggery due to minimal processing during the manufacturing process, whereas in the case of refined sugars, the extensive purification procedures lead to the flushing out of phenolic compounds.[10]

Limitations of the Study

In the present study, the sample size was limited. A larger sample size is required for determining the effect of natural sugar substitutes on salivary pH and *S. mutans* count. Studies with wider age groups, especially preschoolers and children with active carious lesions, need to be done in the population to assess the anticariogenicity of these substitutes. There is a need to study the long-term effects of stevia, jaggery, and honey on oral and general health. Along with salivary pH, the plaque pH changes could have been determined as a carious process resulting from an imbalance in the physiologic equilibrium between tooth and plaque fluid. Further studies are required, including longitudinal investigations to assess the other aspects of caries risk and the side effects of consuming natural sugar substitutes.

Conclusion

Within the limitation of the study, it was concluded that natural sugar substitutes (stevia, jaggery, and honey) have the ability to reduce caries risk in children. However, further research is required before stevia, jaggery, and honey can be used as an adjunct in reducing the risk of caries in an individual.

Clinical Significance

It is a universally known fact that dental caries is a multifactorial disease process, one of the key factors of which is the consumption of sugar. Of all the fermentable carbohydrates, sucrose is considered the archcriminal in the carious process. Hence, this advocates the need for developing suitable sugar substitutes that help in controlling dental caries. An ideal sugar substitute should not only minimize the risk of dental caries but also should have nutritional benefits.

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