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Original Article

Efficacy of Stevioside sweetener on pH of plaque among young adults

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ABSTRACT

Background: Stevioside is a natural herbal sweetener extracted from *Stevia rebaudiana*. An *in vitro* study has proved the antibacterial efficacy of 0.2% *Stevia* aqueous solution against *Streptococcus mutans* and *Lactobacillus acidophilus*, and the present study was conducted to clinically evaluate the efficacy of *Stevia* leaf extract and *Stevia* product on plaque pH, when compared with sucrose solution.

Materials and Methods: A clinical trial was conducted among a sample of 22 undergraduate students who volunteered. After obtaining consent, students were instructed not to brush at night and not to use any mouth rinse during the course of the study. Baseline plaque pH was measured *in situ* using digital pH meter. Students were asked to rinse for 1 min with 0.2% aqueous solution of *Stevia* leaf extract and plaque pH was measured *in situ* at 4 time points (5, 10, 15, and 30 min) after each rinse. After a washout period of 2 days, 10% sucrose and 1% *Stevia* product solutions were similarly tested. Statistical analysis was performed using analysis of variance (ANOVA) test and repeated measures ANOVA. Tukey's HSD test was used to obtain multiple comparisons. The level of significance was set to be at $P < 0.05$.

Results: At 5, 10, 15, and 30 min, a significant difference in mean plaque pH values was observed between three test solutions ($P < 0.000$). *Post hoc* Tukey's HSD test showed that the difference in mean pH values between aqueous *Stevia* extract and sucrose and *Stevia* product and sucrose was highly significant ($P < 0.000$).

Conclusion: *Stevia* leaf extract and commercially available *Stevia* product did not significantly affect plaque pH values, implying that two solutions are non-fermentable and do not support bacterial survival.

Key Words: Dental caries, dental plaque, pH, *Stevia rebaudiana*, sucrose

INTRODUCTION

Dental caries is the most prevalent, ubiquitous infectious disease affecting all the age groups. Fermentable dietary sugar has been implicated as a crucial factor in dental caries and sucrose is an important factor that contributes to the formation and development of the bacterial plaque.[1] Stephan in his classic studies in the early 1940s showed that dental plaque exposed to sucrose could rapidly produce acids, causing a rapid drop in pH followed by a gradual recovery toward the baseline plaque pH.[2] Dental caries despite being preventable continues to be a public health concern in developing countries like India.[3] With support from the evidence, replacement of sucrose with non-fermentable sugar substitute has

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become an important strategy in caries prevention.[4,5] Most of the non-fermentable sweeteners has its own inherent side effects. Long-term consumption of these artificial sweeteners can cause adverse effects in humans, thereby raising health concerns.[6]

The popularity of stevioside as a natural herbal sweetener extracted from *Stevia rebaudiana* (also known as sweet tulsi or sugar leaf) is growing rapidly throughout the world. It is extensively grown in places such as Brazil, Central America, and Israel but is native to Paraguay. It has also been introduced in India since the past decade. Stevioside and rebaudioside A are the most represented glycosides in *S. rebaudiana* leaves which are responsible for sweetening effect of *Stevia*. Stevioside tastes between 200 and 300 times sweeter than sucrose and its content varies between 4% and 20% of the dry weight of the leave, depending on the growing conditions. Rebaudioside A has a clean sweet taste and it is more water-soluble than stevioside; the sweetening power is between 250 and 450 times higher than sucrose.[7-10] *Stevia* extracts are officially approved as food additives in Brazil, Korea, Japan, the United States, and Iran.[11] Besides sweetness, stevioside along with Rebaudioside A offers other therapeutic benefits as they have antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, antidiarrheal, diuretic, and immunomodulatory actions.[12-14]

The antibacterial activity of different extracts of *S. rebaudiana* leaves against bacteria that are important in dental caries and oral health has been proved in *in vitro* studies. Das *et al*., 1992 in an *in vitro* study proved the noncariogenic potential of stevioside.[15] Mohammadi-Sichani *et al*., Debnath *et al*., Gamboa and Chaves, and Ajagannanavar *et al*. demonstrated the antimicrobial activity of *Stevia* in various solvents against *Streptococcus mutans* and *Lactobacillus acidophilus*.[11,16-18] The noncariogenic potential of *Stevia* extracts – stevioside and rebaudioside *in vivo* – was proved in a study conducted by Brambilla *et al*. in 2013.[19] The antiplaque and antigingivitis properties of *Stevia* have been reported by Vandana *et al*.[20] However, no studies on the effect of aqueous solution of *Stevia* leaf extract and commercially available *Stevia* product on plaque pH are available. The antibacterial efficacy of 0.2% aqueous solution of *Stevia* leaf extract against *S. mutans* and *L. acidophilus* was studied *in vitro* in the Department of Public Health Dentistry, FDS, MSRUAS. However, the antibacterial efficacy of commercially available *Stevia* product could not be assessed, and hence, we decided to further explore it clinically. The objective of this study was to evaluate clinically the effect of *Stevia* leaf extract and *Stevia* product on plaque pH when compared with sucrose solution.

MATERIALS AND METHODS

This was an interventional study with 22 participants carrying out mouth rinsing with different solutions such as 0.2% aqueous *Stevia*, 10% sucrose, and 1% *Stevia* product. Study participants were the student volunteers of Bachelor of Dentistry, aged between 18 and 25 years. The study proposal was drafted and the ethical clearance was obtained from the Institutional Ethics Committee and registered at http://www.ctri.nic.in (CTRI/2017/10/010154).

Sample size calculation

Based on the previous study, the observed mean difference was calculated to be 0.5.[17] Assuming the superiority margin of 0.4 with an effect size of 0.53, power 80%, and alpha error 5%, a sample size of 22 was calculated using nMaster sample size software version 2.0. (Department of Biostatistics, Christian Medical College, Vellore, India).

Potential participants for this study were identified from undergraduate students and a complete dental examination was performed. The inclusion criteria were students aged between 18 and 25 years and decayed, missing, and filled teeth (DMFT) score ≥1. Students were excluded if they were undergoing orthodontic treatment or with a history of taking antibiotics within 4 weeks and during the study period.

Preparation of rinses

The antibacterial efficacy of 0.2% aqueous solution of *Stevia* leaf extract against *S. mutans* and *L. acidophilus* was studied *in vitro* in the Department of Public Health Dentistry, FDS, MSRUAS. In disc diffusion method, minimal inhibitory concentration (MIC) of aqueous *Stevia* extract against *S. mutans* was determined at 2 mg/ml concentration. Based on this finding, the following rinsing solutions were prepared as follows. Rinse 1: Aqueous *Stevia* solution was prepared by dissolving 0.2 g of the dried *Stevia* leaf powder in 100 ml of distilled water and brought to boil at 50°C for 2 min and filtered (0.2% aqueous *Stevia* solution).Rinse 2: Sucrose test solution – considering the sweetness equivalence, it was prepared by dissolving 10 g of sucrose in 100 ml of distilled water (10% sucrose solution).[21] Rinse 3: *Stevia* product

solution was made from Cerovia manufactured by *Stevia* world. Cerovia powder is 10 times sweeter than sucrose. Adjusting the sweetness equivalence, the solution was prepared by dissolving 1 g of Cerovia powder in 100 ml of distilled water (1% *Stevia* product solution).

Study protocol

This interventional study was conducted over a period of 2 months. Having given informed consent, 22 volunteers fulfilling the inclusion criteria were recruited for the study. The purpose of the study was explained to the recruited study participants. They were instructed not to brush at night and not to use any mouth rinse during the course of the study. A structured proforma was designed to record information on demographic characteristics, oral hygiene practices, and sugar intake. Clinical examination was carried out using autoclaved instruments. DMFT index and Silness and Loe plaque index (1964) were recorded at the baseline. The students were instructed not to drink or eat for at least 2 hrs before pH measurements.

Plaque pH measurement

Baseline plaque pH was measured by a microelectrode attached to a digital pH meter (LUTRON PH-206). pH microelectrode was inserted at interproximal site between first molar and second premolar in first and second quadrant (16, 26).[22] In case of the presence of any restoration, measurements were done in the first and second premolars interproximal area of the same quadrant. The pH value was recorded by placing the tip of the electrode into the plaque mass and held in place until the reading on the display unit had stabilized and the data were recorded.

Instrument calibration and standardization

Initially, the tip of new pH electrode was soaked in KCl solution for several hours before use. Once prepared, the electrode was stored in a reference buffer (pH = 7). Immediately before and after each series of readings at each time point, the electrode was calibrated against standard pH buffers at pH 4 and 7 values. Between each reading, the electrode was cleaned in distilled water and dried on absorbent paper to protect against cross-contamination.

For each subject, baseline plaque pH was recorded and followed by 5, 10, 15, and 30 min interval after 1 min rinsing of 10 ml of the test solutions. After measuring the baseline plaque pH, all the students were given 10 ml of 0.2% aqueous *Stevia* solution. They were asked to rinse for 1 min. Quantity of rinses was measured using a measuring cup and the time was noted using a stopwatch. Plaque pH was measured at 5, 10, 15, and 30 min after the mouth rinse using the digital pH meter. One examiner performed all pH measurements who was blinded with respect to the rinse used by the students. A washout period of 2 days was given to avoid the carryover effect of the mouth rinse before the next mouth rinse is assigned. After the washout period, the second solution and third solutions were similarly tested [Figure 1].

Statistical analysis

Data were analyzed using SPSS version 16.0. (IBM Corporation, Chicago, IL, USA). For comparison of mean pH values of different times within aqueous *Stevia* extract, sucrose, and *Stevia* product groups, repeated measures analysis of variance (ANOVA) test was used. ANOVA test was used to compare the mean pH values between aqueous *Stevia* extract, sucrose rinses, and *Stevia* product. This was followed by *post hoc* Tukey's HSD test to obtain multiple comparisons. The level of significance was set to be at $P < 0.05$.

RESULTS

Twenty-two volunteers took part in the study, 14 of which were female and eight were male.

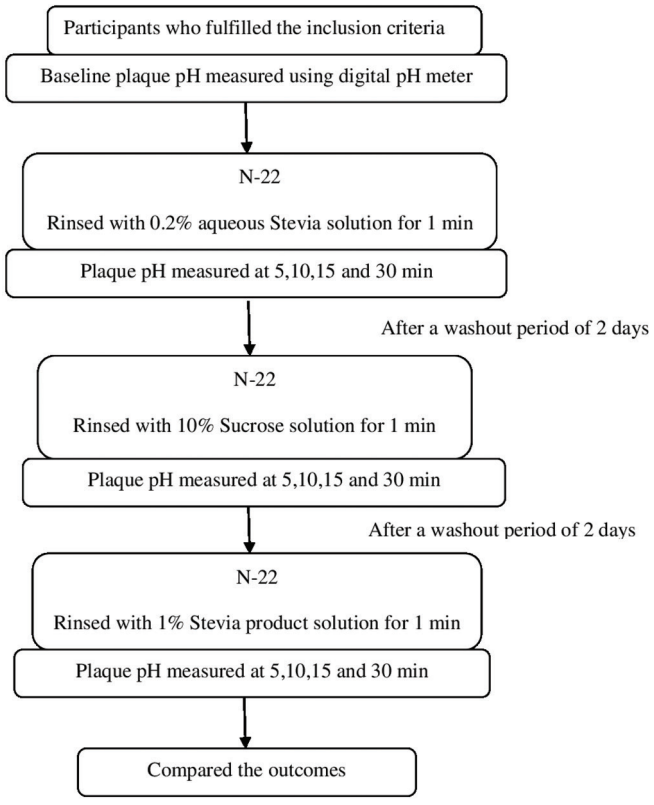

Figure 1: Schematic representation of study design.

Mean DMFT and plaque index score were 2.09 and 0.507, respectively.

Repeated measures ANOVA showed a significant difference in the mean pH values of aqueous *Stevia* extract at 5 ($P = 0.003$) and 10 ($P = 0.024$) min when compared with baseline, whereas mean plaque pH of sucrose solution showed statistically significant difference at 5, 10, 15, and 30 min compared to baseline pH ($P < 0.000$). No statistically significant difference in pH was observed at 5, 10, 15, and 30 min with *Stevia* product when compared to baseline pH ($P > 0.05$).

ANOVA was used to compare the mean plaque pH values between aqueous *Stevia* extract, sucrose rinses, and *Stevia* product. At the baseline, there was no statistically significant difference in mean plaque pH values between the three test solutions ($P = 0.314$), whereas at 5, 10, 15, and 30 min, statistically significant difference in mean plaque pH values was observed between three test solutions ($P < 0.000$) [Table 1]. *Post hoc* Tukey's HSD test [Table 2] showed that the difference in mean pH values between aqueous *Stevia* extract and *Stevia* product was not statistically significant ($P > 0.05$). However, the difference between aqueous *Stevia* extract and sucrose and *Stevia* product and sucrose was highly significant ($P < 0.000$).

Figure 2 illustrates the reduction in mean plaque pH at 5, 10, 15, and 30 min interval after rinsing of three test solutions. Following sucrose rinse, the plaque pH decreased up to 5.7 nearing the critical pH value (5.5) after 5 min, whereas the plaque pH remained almost the same after rinsing with *Stevia* leaf extract and *Stevia* product solutions.

Table 1: Comparison of mean plaque pH values between three groups at five time points

Time points	Plaque pH values Mean±SD			ANOVA	
	Stevia leaf extract	Sucrose	*Stevia* product	*F*	*P*
0 min	7.273±0.28	7.127±0.41	7.144±0.32	1.181	0.314
5 min	7.144±0.25	6.019±0.28	7.055±0.37	89.4	0.000*
10 min	7.150±0.35	5.619±0.31	7.030±0.35	139.3	0.000*
15 min	7.222±0.46	5.677±0.31	7.035±0.36	105.9	0.000*
30 min	7.212±0.41	6.150±0.27	7.075±0.27	69.05	0.000*

*P<0.000. SD: Standard deviation; ANOVA: Analysis of variance

DISCUSSION

The present study was conducted to evaluate the clinical efficacy of 0.2% aqueous solution of *Stevia* leaf extract and commercially available *Stevia* product on plaque pH, in comparison with sucrose solution. The findings showed that there was a reduction in the mean plaque pH following rinsing with 10% sucrose solution whereas the plaque pH remained almost the same after rinsing with *Stevia* leaf extract and *Stevia* product solutions. There was no statistical difference in the pH values among the students at baseline. The change in plaque pH values after rinsing with *Stevia* leaf extract and *Stevia* product solution is consistent with the findings of Brambilla *et al*., who investigated the effect of the two main *Stevia* extracts, stevioside, and rebaudioside A on plaque pH and reported that the two compounds do not support acidogenic metabolism from supragingival plaque bacteria. The probable mechanism of action could be due to an inhibitory effect of the two *Stevia* extracts on

Table 2: Intergroup comparison of plaque pH

Dependent variable	Group		SE	*P*	95% CI	
					Lower bound	Upper bound
pH 5	*Stevia* extract	Sucrose	0.09353	0.000*	0.9005	1.3495
	Sucrose	*Stevia* product	0.09353	0.000*	−1.2604	−0.8114
	Stevia extract	*Stevia* product	0.09353	0.609	−0.1354	0.3136
pH 10	*Stevia* extract	Sucrose	0.10203	0.000*	1.2865	1.7763
	Sucrose	*Stevia* product	0.10203	0.000*	−1.6563	−1.1665
	Stevia extract	*Stevia* product	0.10203	0.472	−0.1249	0.3649
pH 15	*Stevia* extract	Sucrose	0.11582	0.000*	1.2670	1.8230
	Sucrose	*Stevia* product	0.11582	0.000*	−1.6357	−1.0797
	Stevia extract	*Stevia* product	0.11582	0.246	−0.0907	0.4653
pH 30	*Stevia* extract	Sucrose	0.09829	0.000*	0.8259	1.2977
	Sucrose	*Stevia* product	0.09829	0.000*	−1.1605	−0.6886
	Stevia extract	*Stevia* product	0.09829	0.349	−0.0986	0.3732

Multiple comparison Tukey's HSD test;*P<0.000 Significant. CI: Confidence Interval; SE: Standard Error

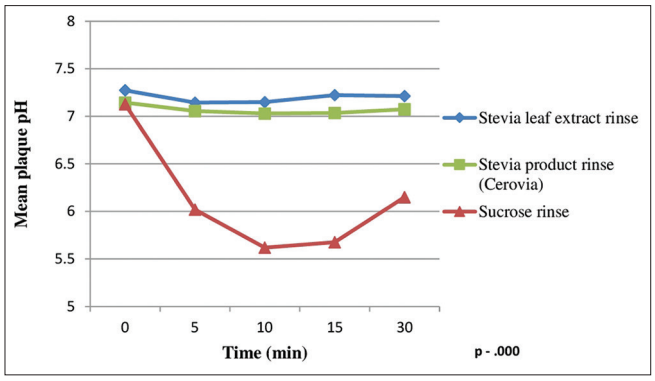

Figure 2: Mean plaque pH versus time curves for all test solutions.

bacterial fermentative metabolism. *In vitro* part of this study confirmed the cariostatic potential of the *Stevia* extracts by the suppression of bacterial growth.[19]

In the present study, 0.2% concentration of aqueous *Stevia* extract was prepared. This was based on the findings of an *in vitro* study conducted in our department. In disc diffusion method, MIC of aqueous *Stevia* extract against *S. mutans* and *L. acidophilus* was determined at 2 mg/ml concentration. The antibacterial efficacy of *Stevia* product could not be proved in the *in vitro* study, and we decided to further explore it clinically. Considering the sweetness equivalence, sucrose and *Stevia* product solutions were prepared.

Various methods have been used by different investigators to determine the pH of dental plaque of which each method has its strength and weakness.[23,24] In our study, the method used for this purpose was single-glass electrode fitted to a digital pH meter (LUTRON PH-206) which allows direct reading of interdental plaque pH. The trial was a single-blinded trial as the examiner who measured the plaque pH was blinded with respect to the rinse used by the students.

On the basis of *in vitro* experiments and theoretical considerations, critical pH has been reported to be in the range of 5.0–6.0, most probably 5.5. In this study, no plaque pH drop below 5.5 (critical pH) was recorded following sucrose rinse group due to methodological issues. All the participants in the study were dental students and seem to have better oral hygiene practices. Various researches have proved that mature plaque (2–3 days old) give a greater level of acid production than immature plaque. Moreover, the pH fall itself depends upon various factors such as acidogenicity of the plaque microflora, nature of the acids formed, formation of neutralizing metabolic products, buffering capacity of the plaque, concentration of substrate surrounding the bacteria, and duration of the supply of the substrate, diffusion of substrate and metabolic products in plaque, influence of the saliva environment of these parameters.[25]

In this study, mean plaque pH of aqueous *Stevia* extract showed significant difference at 5 and 10 min when compared with baseline. The plaque pH remained alkaline throughout different time intervals for both *Stevia* leaf extract and *Stevia* product solution. Although the antibacterial efficacy of commercially available *Stevia* product could not be proved in the *in vitro* study, clinically, it behaved in a similar way to aqueous *Stevia* extract solution. Most commercial processes consist of water extraction, decoloration, and purification using ion exchange resins, electrolytic techniques, or precipitating agents. The possible reason for this activity needs to be explored. Due to the nonavailability of the evidence, the results of commercially available *Stevia* product in altering plaque pH could not be compared. In 2017, Usha *et al*. proved that 0.5% *S. rebaudiana* extract improved the pH and buffering capacity of the saliva in a high caries risk patient.[26] Abdul Razak *et al*. had reported that alternative sweeteners such as equal *Stevia* were equally effective as xylitol in reducing the presence of extracellular matrix in streptococci biofilms.[27] This study mainly concerns with its influence on change of pH within plaque, and therefore, further researches on microbiological analysis of stevioside on cariogenic species are needed for confirmation.

CONCLUSION

Clinically, both *Stevia* leaf extract and *Stevia* product solutions behave in a similar way as the plaque pH remained alkaline. *Stevia* leaf extract and commercially available *Stevia* product did not significantly affect plaque pH values implying that two solutions are nonfermentable and do not support bacterial survival. It appears to be a promising herbal sweetener to be used as an alternative in oral preparations and confectionaries.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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