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Comparing The Effect Of Natural And Synthetic Sugar Substitutes On Salivary Ph And Streptococcus Mutans Growth - An *In vivo* Study

Research Article

S. Sushanthi[1], Leelavathi. L[2*], Meignana Arumugham. I[3]

[1] Post-Graduate Student, Saveetha Dental College, Saveetha University, Saveetha Institute of Medical and Technical Science, Chennai, India.
[2] Senior Lecturer, Department of Public Health Dentistry, Saveetha Dental College, SIMATS, Chennai- 77, India.
[3] Professor, Department of Public Health Dentistry, Saveetha Dental College, SIMATS, Chennai- 77, India.

Abstract

Background: Nowadays sugar free food are very much popular and Artificial sweeteners have adverse effects when it is taken in more quantity. Currently, Stevia rebaudiana, considered to be a good replacement of sugar which is healthy and have much fewer side effects.
AIM: To compare the salivary pH and streptococcus mutans growth among the participants mouth rinsing water with artificial sweeteners and natural sweeteners.
Materials And Methods: This study was a double blinded parallel invivo study. Forty female participants aged 22-25 years were randomly selected and allocated by lottery method into four different groups as group A, B, C and D. Salivary pH assessments were performed at baseline and after drinking different sugar solutions with aspartame(single tablet and two tablets) and stevia(single tablet and two tablets) mixed in distilled water at 20 minutes and checked for microbial growth. ANOVA and paired t test were used to analyze the data.
Resuls: The results show that mean salivary pH of group B(0.000) and C(0.004) and mean streptococcus mutans count among all the groups have statistically significant difference. Group C have low streptococcal mutanscount(1.9±0.38 × 103) and stable pH value(7.04±0.10). Group B have high mutans count (2.4±0.65 × 103) and low pH (6.96±0.24) value among all the groups.
Conclusion: After mouthrinsed with Stevia, salivary pH came back to neutral state and it has showed low streptococcus mutans growth compared with aspartame groups . This indicates that stevia can act as good natural sugar substitute.

Keywords: Salivary pH; Aspartame; Stevia; Sugar Substitutes; Mouth Rinsing.

Introduction

Sweeteners are additives which gives us prime taste of sweetness to a food product. Commonly sugars are used as sweeteners in food and it provides energy of 4kcal/g in addition to the taste. But increasing obesity rates tells us to eschew over consumption of calories. Nowadays people are usually health conscious and this led to a predominant rise need for low calorie fat products [1].

Stevia is a plant native to South America which has been used as a sweetener for hundreds of years. Presently zero calorie stevia which has high naturalness of stevia extract is being used worldwide to decrease calorie content and added sugar content in foods and drinks. Stevia is the common term used to mention many forms of the sweetener which contain theplant Stevia (S rebaudiana Bertoni) and the leaves which are sweet parts of the plant. The sweet-tasting constituent of stevia are known assteviol glycosides, which are obviously present in the stevia leaf. Refined stevia leaf extracts have one steviol glycoside or many different glycosides, which can be almost 250 to 300 times sweeter than sucrose [2].

Artificial sweeteners which are known as sugar substitutes, alternative sweeteners, or non-sugar sweeteners, are substances used to replace sugar in foods and beverages. They can be separated into two huge divisions namely nutritive sweeteners, which in-

***Corresponding Author**:
Dr. Leelavathi.L., M.D.S.,
Associate Professor, Department of Pedodontics and Preventive Dentistry, Sri Sai College of Dental Surgery, Vikarabad, Telangana, India.
Tel: 8220870849
E-mail: karleela81@gmail.com

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crease some energy (calories) to food; and non-nutritive sweeteners, which are also known as high-intensity sweeteners because they are used in very minute quantities, which has nil energy components to food [3]. Aspartame which is discovered in 1965, is a low-calorie sweetener which has a very sweet taste and it is almost 200 times sweeter than sucrose [4, 5]. Theworld population consumes about 2000 tonnesyearly of aspartame, which is an artificial sweetener, has two amino acids—aspartic acid and phenylalanine [6].

Salivary pH shows the hydrogen ion concentration present in saliva which gives us information about its acidic and alkaline nature. Chloride ion is higher in nonstimulated saliva or when flow is low which leads to low pH which takes to less buffer. Diurnal changes affect the buffering capacity of saliva, usually high in the morning [7].

Presently sugar free foods are more accepted because of their less calorie content. The intake of sugar substitute in food started to showcase the decline of prevalence of dental caries in developing countries [8]. sucrose substitutes have good sweetness but they do not undergo metabolic activity in the body and therefore they don't give us calorie intake [9].

The literature search shows us that less studies are done to assess the salivary pH change and between artificial and natural sweetener. This study is to assess and to evaluate the difference in salivary pH and streptococcus mutans count after consuming artificial and natural sweeteners. In this study we have taken Aspartame (sugar gold) as artificial sweetener and Stevia as natural sweetener and planned to compare the difference in salivary pH after rinsing with both sugar solutions and to compare the streptococcus mutans count in saliva before and after rinsing with the sugar solutions. We have planned to assess the salivary pH difference before and after consuming artificial and natural sweetener and also difference in streptococcus mutans count before and after consuming artificial and natural sweetener respectively.

Materials And Methods

Study design

It is a double blinded, parallel, in vivo study

Study population

Interns of Saveetha dental college and hospital, Chennai were selected for this study.

Groups:

Group A - 30ml of distilled water with one tablet of Sugar free Gold (Aspartame).
Group B - 30ml of distilled water with two tablets of Sugar free Gold (Aspartame).
Group C - 30ml of distilled water with one tablet of Stevia.
Group D - 30ml of distilled water with two tablets of Stevia.

Random allocation through lottery method was done by a separate person who was not involved in the study for allocation of participants into the above-mentioned groups.

Eligibility criteria:

Inclusion criteria:

Study participants with normal healthy gingiva.
Healthy study participants without any systemic illness above 18-24 years of age.
Dental students with habit of tooth brushing twice daily.

Exclusion criteria:

Study participants who were using antisialagogues or drugs that reduce salivary flow rate.

Study participants who were undergoing orthodontic treatment.
Study participants with dental caries affecting not more than one tooth.

Sample size determination:

Sample size was calculated using priori by G*power 3.1.2 software. According to Tanushri MD et al, the minimum sample size of each group was calculated. Following these input conditions: Power of 0.95 and P≤0.05 and sample size we got was 10 per each group.

Ethical clearance:

Ethical clearance was acquired from the institutional ethics committee, Saveetha University (IHEC/SDC-PHD-1901/20/254).

Blinding:

Participants and investigators were unaware about the groups of the allocation.

Consent:

Voluntary informed consent was got from the study participants before to the start of the study.

Preparation of the test solutions:

The test solutions were prepared by using commercially available sugar substitutes which are Sugar free gold containing Aspartame, Stevia in form of tablets. Required tablets were added to the 30ml of distilled water according to the groups divided and stirred for 10 seconds till the tablet gets completely dissolved in it.

Intervention details

Once the students were selected, they were randomly distributed to different groups and their unstimulated salivary sample were collected at the baseline and pHwas determined with the help of salivary pH indicator strips. After determination of baseline pH, the subjects were instructed to mouthrinse with the solutions at least for 30 seconds [10] by swishing the entire content in the mouth at once and expectorate after which the unstimulated salivary pH was again assessed at 20 minutes [11] respectively. Figure 1 shows the pH strips colour changing when immersed in saliva.

Method of saliva collection

All subjects were given transparent instructions to restrict from eating for one hour before collection of saliva. The subjects were instructed to let saliva pool in the floor of the mouth for at least 1 minute and then expectorate in the uricolbox [12]. Figure 2 shows the collected saliva before and after consuming sweeteners which is kept in order.

Salivary pH estimation

Salivary Ph was checked using dental salivary pH indicator strips. The pH strips were dipped into the collected saliva and taken out immediately and observed for 10 seconds for the colour change. The alteration in colour was analyzed with the reference given by the manufacturer and readings wereentered [13]. Salivary Ph was recorded at baseline and then after mouth rinsing with the sugar substitute containing solutions at 1 minute, 20 minutes respectively.

Microbial growth estimation

Sanguismutans agar medium was prepared and sterilized. After sterilization the prepared media was poured on to the sterile petri plates and kept for solidification. After solidification collected saliva from the participants is taken in a cotton swab and swabbed over the petri plates. Then it is kept for incubation at 37degree Celsius for 24 hours. Figure 3,4,5 and 6 represents streptococcal mutans growth after incubation for Group A,B, C and D respectively.

Statistical analysis

The data obtained during the course of the study was systematically entered in Microsoft Excel sheet. Data analysis was performed using SPSS software version 20. Data was normally distributed, so parametric tests have been employed. One-way analysis of variance was employed to compare the means of salivary pH and Streptococcus mutans count between the groups. Paired t test was done to compare the mean salivary pH and streptococcus mutans count within the group.

Results

There was a predominant difference in mean salivary pH between the groups after mouth rinsing with solutions containing one tablet Aspartame (group A), two tablets of Aspartame (group B),

Figure 1. pH strips after dipping with saliva.

Figure 2. Uricol boxes with saliva collected for Group 1 Study participants.

Figure 3. Group A S.mutans growth after incubation.

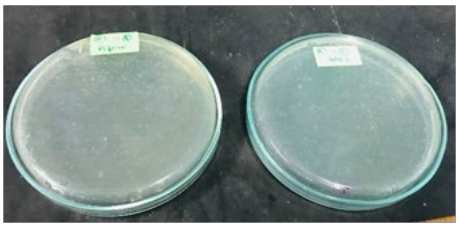

Figure 4. Group B S.mutans growth after incubation.

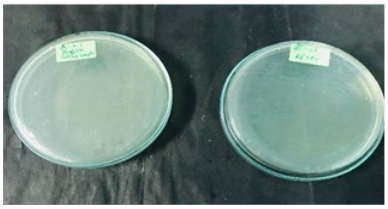

Figure 5. Group C S.mutans growth after incubation.

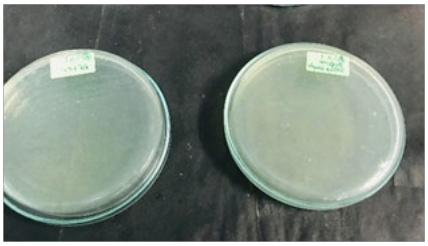

Figure 6. Group D S.mutans growth after Incubation.

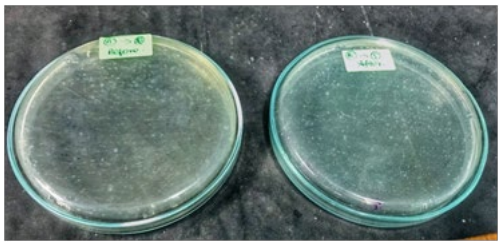

Figure 7. Flowchart Representation.

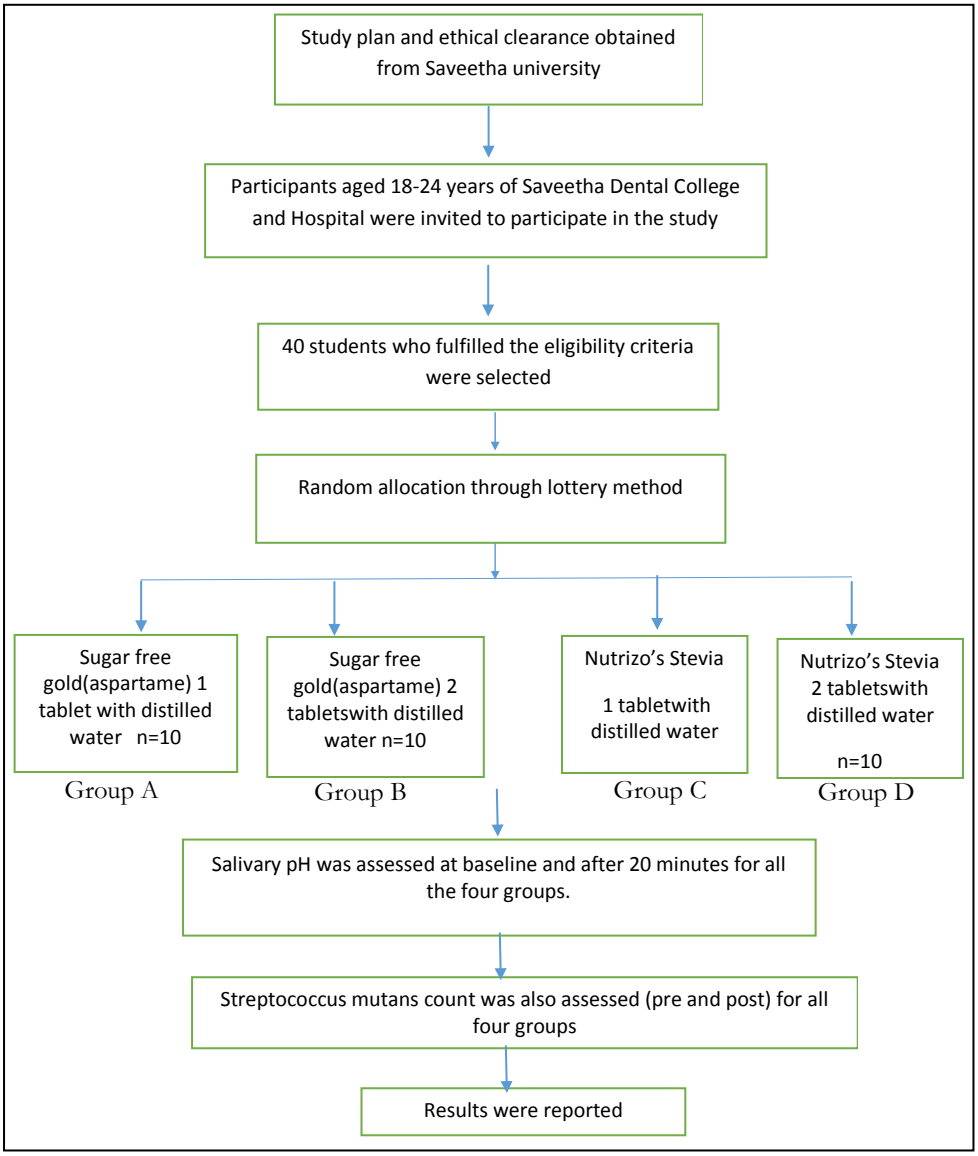

Figure 8. Depicting the salivary pH values of four groups in a bar graph.

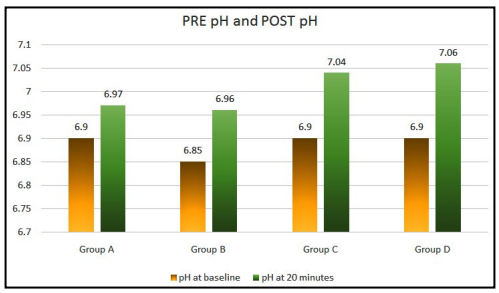

Figure 9. Depicting the Streptococcus mutans count of study participants in a bar graph.

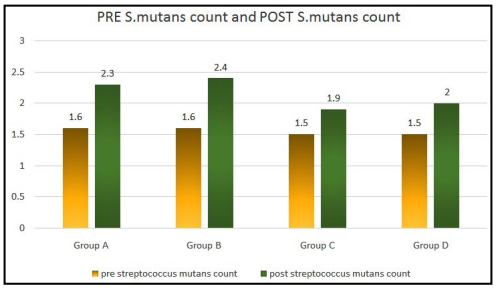

Table 1. Mean distribution of salivary pH and comparison of salivary pH among and between the groups.

Interventional groups	N	Mean salivary pH and standard deviation at baseline	Mean salivary pH and standard deviation at 20 minutes	Mean difference between pH	P value within the groups
Group A (single tablet aspartame)	10	6.9 ± 0.16	6.97 ± 0.15	0.07	0.111
Group B (two tablets Aspartame)	10	6.8 ± 0.27	6.96 ± 0.24	0.16	0.000*
Group C (single tablet stevia)	10	6.9 ± 0.17	7.04 ± 0.10	0.14	0.004*
Group D (two tablet stevia)	10	6.98 ± 0.18	7.06 ± 0.11	0.08	0.104
P value between the groups	0.551	0.449			

p value –probability value
* Statistically significant at $p < 0.05$ (2-tailed).

Table 2. Mean distribution of streptococcus mutans count and comparison of S.mutans count among and between the groups.

Interventional groups	N	Mean streptococcus mutans count and standard deviation at baseline ($\times 10^3$)	Mean streptococcus mutans count and standard deviation at 20minutes ($\times 10^3$)	Mean difference between streptococcus mutans count from baseline to 20 minutes ($\times 10^3$)	P value within the groups
Group A (single tablet aspartame)	10	1.6 ± 0.29	2.3 ± 0.55	0.6	0.001*
Group B (two tablets Aspartame)	10	1.6 ± 0.55	2.4 ± 0.65	0.7	0.001*
Group C (single tablet stevia)	10	1.5 ± 0.32	1.9 ± 0.38	0.4	0.000*
Group D (two tablet stevia)	10	1.5 ± 0.22	2 ± 0.53	0.5	0.002*
P value between the groups	0.619	0.141			

p value –probability value
* Statistically significant at $p < 0.05$

one tablet of Stevia (group C), two tablets of Stevia (group D) at 20 minutes. At baseline mean pH value of all the groups are mostly 6.85- 6.98 (figure 8). At 20 minutes obviously the count increases for all the groups and for groups C and D pH value is around 7.1(figure 8). Among all the groups group C has the lowest streptococcus mutans growth and higher level of pH. Out of 4 groups, group C have the neutral pH whereas Group A and B have their pH value in acidic state. Group C have the lowest streptococcus mutans count (1.9× 10^3 CFU/ml) (figure 9) and group B have the highest streptococcus mutans count (2.4× 10^3 CFU/ml). ANOVA test reveals that there was no statistically significant difference between the groups for pH count and streptococcus mutans count. Paired sample test showed that for pre pH and post pH values group B (0.000) and group C (0.004) shows statistically significant difference(table 1) and for pre mutans and post mutans value all the groups shows statistically significant difference(table 2). There were no adverse effects or harmful outcomes occurred during and after the study.

Discussion

The current study analyzed and compared the baseline salivary pH and alterations after mouthrinsing with Aspartame(artificial sweetener) and Stevia(natural sweetener) in different quantity of tablets after 20 minutes. To the author's knowledge this is the first study to compare the salivary pH changes and streptococcus mutans growth changes between artificial and natural sweeteners. The results shows that there is a slight increase in rise of pH value in group C(Stevia). This results is in agreement with the study results of Goodson J et al [14], when compared between stevia oral rinse group and sucrose oral rinse group statistically significant rise in plaque pH occurred. In the current study there was significant rise in salivary pH in all the groups after mouth rinsing with the respective sugar solutions from baseline to 20 minutes. A study done by Tanushri MD et al., [15] reveals that there was a significant rise in salivary pH after mouth rinsing with Stevia solution which is in accordance with our current study.

According to Tanushri MD et al., [15], stevia has anti-bacterial activity on Streptococcus mutans, Streptococcus sobrinus which comes under major oral microorganisms which is responsible for dental caries. Hence it can be a perfect sugar substitute and can replace sugar in all situations in a healthy way. According to Motamaye llFA [16] males show low salivary pH than female so to avoid confusions we have selected only female students in our current study. According to Praskevas et al., [17], 30 second of mouth rinsing is more than enough for all the tooth surfaces to come in contact with the solutions.Thus time duration to rinse with solutions is finalized for 30 seconds. Stimulated saliva shows variations in salivary pH but unstimulated saliva shows basal salivary flow rate and it does not show considerable variations in salivary pH [18].

According to Maryam et al., [19], stevia have good antimicrobial activity against streptococcus mutans and our current results also depicts the same in which Group C shows less mutans growth compared with other groups.Saira Siraj et al, [20] tells us that commercial available stevia products have good antibacterial activity which correlates with our study result.Thus it can replace artificial sweeteners which is widely in use among the people.

It is shown by several studies that aspartame components causes numerous health problems but it has no correct evidence to support that aspartame causes harm to health and it is considered as safe as nonnutritive sweetener [21]. According to Chatsudthipong V et al [22], stevia sweetener extracts are recommended to exert useful effects on human health, such as anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory effects. According to Blauth de, Slavutzky et al., [23] the major cariogenic organism, S. mutans, has growth suppression and secretes less acid when grown on stevia containing mediacompared with the growth on sucrose, glucose or fructose medium.

Limitations

1. As plaque pH changes are in accordance with salivary pH changes, it would have been good if plaque pH was also determined.

Conclusion

Results of the study showed that among all the groups, study group in which the participants rinsed their mouth with one tablet of natural sweetener(Group C) has the lowest streptococcus mutans growth and highest pH value .Stevia rebaudiana can be an excellent replacement of current artificial sweeteners and can act as a natural and healthy sweetener and helps to overcome the adverse effects causes by artificial sweeteners which is in trend thereby it can also help to overcome the lifestyle associated diseases like dental caries, obesity etc.

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