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Assessing the effect of natural sweetener on salivary pH and streptococcus mutans growth - An invivo study

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ABSTRACT

Stevia is a natural sweetener which is used as a sugar substitute. It has been suggested that Stevia may be anti-cariogenic. However, there is limited research in this regard. Currently, Stevia rebaudiana, a plant is considered to be a suitable replacement of sugar which is healthy and has much fewer side effects than other sweeteners. To assess the salivary pH and streptococcus mutans growth among the participants after mouth rinsing with water containing natural sweetener. This double-blinded parallel clinical trial was done among forty female participants aged 22-25 years. Study participants were selected and randomly allocated by lottery method as two different groups as group A, and B. Microbial growth and pH of the saliva was assessed twice, once before rising with stevia solution and at 20 minutes after rinsing with a sugar solution containing Stevia (single tablet and two tablets) mixed in distilled water. Collected data were analyzed using the paired t-test. It was found from the study that there was an increase in mean salivary pH when compared with the baseline value after rinsing with Stevia. There was an increase in the Streptococcus mutans count after rinsing with Stevia. Increase in streptococcus mutans count was found to be low in Group A than group B. pH value stays in neutral value even after rinsing with a natural sweetener. There is no much difference between Group A and B. Salivary pH after mouth rinsing with Stevia is in a neutral state. It has low Streptococcus mutans growth suggesting that Stevia can also be used as a sugar substitute replacing artificial sugar substitutes.

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INTRODUCTION

Sweetness is the taste that is staunchly identified with love and reward. Indulgence in sweets has been mentioned as a 'universal human weakness.' Carious lesions were less in ancient times but drastically increased in the industrialized world. Epidemiological studies in many parts of the world brace the hypotheses that arise in dental caries were associated with dietary changes. The classical evidence from Vipeholm, Hope wood house and Turku sugar studies has shown clearly the significance of diet in the carious process (Newbrun, 1995). Nowadays,

people are usually health-conscious, and this led to a predominant rise in the need for low-calorie fat products (Martínez-Cervera *et al.*, 2012).

A natural sweetener named Stevia, being used in diabetic patients which is a subject of dental research. It got attention as a useful natural substance to treat a variety of ailments due to its antibacterial and anti fungal properties (Goyal *et al.*, 2010). Stevia is derived from Stevia Rebaudiana plant species and consists of stevioside, rebaudioside A, D and E, dulcoside A and B (Ferrazzano *et al.*, 2015). Stevia is 100% natural, zero calories, 200-300 times sweeter than sugar, heat stable, non-fermentable, and has anti-plaque and anticaries activity. Stevia is available in different forms as Table sugar, drops, hard candy, additives in beverages, dairy products, cakes and confectionery but is also added recently in a mouth rinse, chewing gum and toothpaste (Wald and Morlock, 2017). Stevia was approved to be used as a natural sweetener by the FDA in 2008 (Fitch and Keim, 2012).

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 essential factor that contributes to the formation and development of the bacterial plaque (Whelton *et al.*, 2019). 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 (Amaechi, 2015). Dental caries, being preventable, continues to be a public health concern in developing countries like India (Simratvir *et al.*, 2011). With support from the evidence, replacement of sucrose with non-fermentable sugar substitute has become an important strategy in caries prevention (Burt, 1993; Larmas, 2010). Most of the non-fermentable sweeteners have their inherent side effects. Long-term consumption of these artificial sweeteners can cause adverse effects in humans, thereby raising health concerns (Nabavi *et al.*, 2020).

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 unstimulated saliva or when the 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 (Tandel, 2011).

Studies have evaluated the antimicrobial potential of the extracts of S. rebaudiana against many pathogens (Jayaraman *et al.*, 2008; Kinghorn, 2001; Sedghi and Gholi-Toluie, 2014). Few invitro studies have assessed their antibacterial action on S. mutans (Ajagannanavar *et al.*, 2014; Mohammadi-Sichani, 2012). However, only a few clinical studies have been reported on its impact on the salivary level of S. mutans (Zanela *et al.*, 2002; Kinghorn, 2001). As there is insufficient evidence available regarding S. rebaudiana as an anti-cariogenic substance, this study is framed to assess, and the difference in salivary pH and streptococcus mutans count after consuming natural sweetener. In this study, we have taken Stevia as a natural sweetener and planned to compare the change in pH of the saliva before and after rinsing with Stevia and also to compare the streptococcus mutans count in saliva before and after rinsing with stevia solution.

MATERIALS AND METHODS

The present study was an in vivo study, and participants were selected from a private dental college, Chennai. Study participants with mild to moderate gingivitis and without any systemic illness in the age group of 18-24 years were included in the study.

Study participants who are using antisialogogues or drugs that reduce the salivary flow rate, who are undergoing orthodontic treatment were excluded from the study. Ethical clearance was acquired from the institutional ethics committee. Participants and investigators were unaware of the groups of the allocation since a separate investigator was recruited for allocation of the participants.

Nature of the study was explained to the study participants, and they consented to participate in the study. A minimum sample size of each group was calculated using prior by G*power 3.1.2 software.

Following these input conditions: Power was kept at 0.95, and the sample size we got was 20 per each group. Participants were randomly allocated into two groups by lottery method.

Groups

Group A- One tablet of Stevia dissolved in 20ml of distilled water.

Group B- Two tablets of Stevia dissolved in 20ml of distilled water.

Preparation of stevia solution

Commercially available sugar substitutes, Stevia, which is available in the markets in the form of tablets were used for preparing stevia solutions.

Stevia tablets were added to the 20 ml of distilled water according to the groups divided and stirred for 10 seconds till the tablet gets completely dissolved in it.

Table 1: Mean distribution of salivary pH

Interventional groups	N	Salivary pH (base line) Mean ± standard deviation	Salivary pH (After 20 minutes) Mean ± standard deviation	Mean difference between pH
Group A (single tablet stevia)	20	6.9±0.17	7.04±0.10	0.14
Group B (two tablet stevia)	20	6.98±0.18	7.06±0.11	0.08

Table 2: Mean distribution of streptococcus mutans count

Interventional groups	N	Streptococcus mutans count ($\times 10^3$) at base line Mean ± standard deviation	Streptococcus mutans count ($\times 10^3$) at 20 mts Mean ± standard deviation	Mean difference between strepto coccus mutans count ($\times 10^3$)
Group A (single tablet stevia)	20	1.5±0.32	1.90±0.38	0.4
Group B (two tablet stevia)	20	1.50±0.22	2.0±0.53	0.5

Table 3: Comparison of mean salivary pH among study groups.

Interventional groups	Mean ± Standard deviation at base line	Mean ± Standard deviation at 20 minutes	P-value within the groups
Group A (single tablet stevia)	6.90±0.17	7.04±0.10	0.004*
Group B (two tablet stevia)	6.98±0.18	7.06±0.11	0.104
P-value between the groups	0.551	0.449	

*** indicates statistically significant at p < 0.05 (2-tailed).**

Table 4: Comparison of mean streptococcus mutans count among study groups.

Interventional groups	Mean ± Standard deviation at baseline ($\times 10^3$)	Mean ± Standard deviation at 20 minutes ($\times 10^3$)	p-value within the groups
Group A (single tablet stevia)	1.5±0.32	1.90±0.38	0.000*
Group B (two tablet stevia)	1.50±0.22	2.0±0.53	0.002*
P-value between the groups	0.619	0.141	

*** indicates statistically significant at p < 0.05**

Intervention details

The students were randomly distributed to two groups. One group received stevia solution containing one tablet, and the other group received stevia solution containing two tablets. Their salivary pH was estimated before and after administering the stevia solution using pH strips. Once pH of the saliva at baseline was recorded, study participants were asked to rinse with the prepared stevia solutions for 30 seconds in such a way that the entire mouth is rinsed with the stevia solution[10] and they were instructed to expectorate. A pH of the saliva was again recorded after 20 minutes of expectoration (Azrak, 2008).

Procedure for salivary sample collection

Study participants were asked not to eat for one hour before the salivary collection and also during the intervention period. The participants were informed to allow saliva to get pooled in the floor of the mouth for at least 1 minute, and then they were asked to expectorate in the uricol box (Kipps *et al.*, 1975).

Estimation of pH of saliva

pH indicator strips were used to check the pH of the saliva for the study participants. Saliva was collected in an uricol container, and pH strips were dipped into the collected saliva, and color change was checked. pH was assessed using the color coding given by the manufacturer. (Kipps *et al.*, 1975; Animireddy *et al.*, 2014). The pH of the saliva was recorded twice, once before rising with stevia solution and at 20 minutes after rinsing with stevia solution (single tablet and two tablets).

Estimation of Microbial growth

Sanguis mutans agar medium was prepared and sterilized. 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 and kept for incubation at 37degree Celsius for 24 hours.

Statistical analysis

The data obtained were entered into the Microsoft Excel sheet. Data analysis was performed using SPSS software version 20. Parametric tests have been employed as the data was normally distributed. The paired t-test was done to analyze the difference in the mean salivary pH, and streptococcus mutans count within the groups.

RESULTS AND DISCUSSION

An invivo study was done among forty female study participants. The average age of subjects was 20.2 years. There was a predominant difference in mean salivary pH between the groups among one tablet of Stevia (group A) and two tablets of Stevia (group B) at 20 minutes. At baseline mean pH value of all the groups is mostly 6.85- 6.98. At 20 minutes, the value increases for groups A and B, pH value is around 7.2 (Table 1).

Among all the group's group A has the lowest streptococcus mutans growth. From results obtained for both the groups, group A and B have a neutral pH, whereas Group A has the lowest streptococcus mutans count (1.9×10^3CFU/ml) (Table 2). Paired sample test shows that for pre pH and post pH values group A (0.004) shows a statistically significant difference. In contrast, for Group B, it does not show any statistically significant difference (0.104) (Table 3).

Paired T-test for streptococcus mutans count within the groups (pre mutans and post mutans) value of all the groups shows a statistically significant difference (Table 4). There were no adverse effects or harmful outcomes that occurred during and after the study.

A renewed interest has occurred in the last decade to search for antibacterial activity and phytochemicals of native plants (Mohana *et al.*, 2015). S. rebaudiana belonging to the family Asteraceae is a natural alternative to artificial sweetener. It contains over 100 phytochemicals (Idrees *et al.*, 2018). S. rebaudiana leaf extracts demonstrated antibacterial activity. So the study was aimed to assess Stevia's activity with streptococcus mutans and to assess the pH of Stevia with different concentrations. This study reveals the mouth rinsing with stevia pH of the saliva did not drop below 7 (acidic state). This is promising finding as an acidic state of the oral cavity is one of the reasons for more dental caries as the oral microorganisms grow well in this state.

Under resting conditions without the exogenous stimulation that is linked with feeding, there is a steady flow of saliva which keeps the mouth moist and lubricates the mucous membrane (Hegde, 2016). Unstimulated saliva is essential for the health and well-being of the oral cavity and also bestows a strong protective effect on the oral cavity, against dental caries (Kedjarune *et al.*, 1997). The normal pH of saliva is 6.7–7.4, but as bacteria break down carbohydrates, they release lactic acid, butyric acid, and aspartic acid, which bring down the pH of saliva. Since stevia and related products fall shy

of a pH of 7, these are neutral in acidity and are generally recognized as safe by the FDA. According to Singh et al. (Sharma *et al.*, 2017; Singh *et al.*, 2017), it was reported that stevia act against Streptococcus mutans which is one among dental caries causing microorganisms According to Mohammadi-Sinchani et al. (Mohammadi-Sichani, 2012), Stevia has good antimicrobial activity against streptococcus mutans and our current results also depict the same in which Group C shows fewer mutans growth compared with other groups. Hence, it can be a perfect sugar substitute and can replace sugar in all situations in a healthy way. From the study results, there is no predominant increase in the streptococcus mutans count in both the groups when compared with baseline and after twenty minutes, and they show a statistically significant difference in both the groups on paired t-test. In the present study, only salivary pH changes were assessed, which could be a possible limitation as pH changes in plaque could also have been assessed.

CONCLUSION

From the current study findings, it was concluded that after mouth rinsing with Stevia, salivary pH was in a neutral state, and it does not drop to an acidic state. Also, there was a minimum rise in the streptococcus mutans count found after 20 minutes. It implies that stevia rebaudiana could be an excellent replacement of artificial sweetening agents. It can act as a natural and healthy sweetener and assist in overcoming the adverse effects caused by artificial sweeteners which are in trends.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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