ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

EFFECT OF 4.7% STEVIA SOLUTION MOUTH RINSING ON SALIVARY PH: AN IN-VIVO RANDOMIZED CONTROLLED TRIAL

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Article info

Article History:
Received: 01-12-2021
Revised: 20-12-2021
Accepted: 02-01-2022

KEYWORDS:
Stevia, Mouth rinse, Saliva, Dental caries.

ABSTRACT

Background: Stevia is a natural, non-caloric sweetener with antimicrobial, antioxidant and anti-cariogenic properties. Aim was to assess and compare the effect of 4.7% stevia solution, 4.7% sucrose solution and distilled water mouth rinsing on salivary pH. **Methods:** Randomized controlled trial with Latin square design was followed involving 15 female participants aged 20-23 years. Participants were randomly allocated to three interventional groups; **Group A:** 4.7% stevia mouth rinse, **Group B:** 4.7% sucrose mouth rinse and Group C: distilled water mouth rinse. Salivary pH assessments were done at baseline and 1, 20 and 60 minutes post intervention using salivary pH indicator strips. For statistical analysis, significance level was fixed at $p<0.05$. One way and repeated measures ANOVA followed by Tukey's post Hoc tests were used for data analysis Results: There was a significant ($p<0.05$) increase in salivary pH post rinsing with stevia solution (at 1, 20 and 60 minutes respectively) compared to sucrose solution and distilled water mouth rinsing **Conclusion:** Stevia solution mouth rinsing showed significant increase in salivary pH at one hour from baseline compared to sucrose and distilled water mouth rinsing. Hence, it may serve as an anti-cariogenic sugar substitute.

INTRODUCTION

A dynamic relation exists between sugars and oral health. After being hydrolysed by salivary amylase, sugars and other fermentable carbohydrates provide a substrate for oral bacteria to work on, lowering plaque and salivary pH.[1] As a result of this activity, tooth demineralization begins, which contributes to dental caries formation. Hence, one of the areas of interest in preventive dentistry is sugar substitutes which can prevent dental caries but there are various disadvantages associated with utility of sugar substitutes which includes systemic problems related to body weight, incompatibility with cooking of food and unpalatable flavor.[2]

Access this article online	
Quick Response Code	
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With the increasing prevalence of diabetes and obesity and also because of the growing concern for the safety of some chemical sweeteners such as aspartame, cyclamate, saccharin, sucralose, the need for natural non caloric sweetener with acceptable taste and safety is exigent.[3] *Stevia rebaudiana* bertoni is a natural plant derived non caloric sweetener, which has been tested for antimicrobial, antidiabetic, antioxidant, anti-carcinogenic and immunomodulation effects.[4] A systematic review done by Ferrazzano GF *et al,* highlighted the anti-cariogenic properties of stevia and suggested conduct of in-vivo trials to explore this further.[5] Since very few studies have tapped the anti-cariogenic potential of stevia, a trial was designed to test the effect of 4.7% stevia solution mouth rinsing on salivary pH in vivo. The study tested the null hypothesis that there is no difference in the effect of 4.7% stevia solution, 4.7% sucrose solution and distilled water mouth rinsing on salivary pH at one hour post mouth rinsing.

MATERIALS AND METHODS

The study design was experimental, in vivo, cross over, latin square design. The clinical trial

followed the Consolidated Standards of Reports (CONSORT, 2010) guidelines. (Figure 1) Study was approved by Institution Ethical review board of the college where the study was conducted. [Reference NO: BDC/Exam/256/2014-15]. The study was approved and funded by Indian Council of medical research [ICMR, STS- Ref ID: 2014-04305]. Sample size was calculated using G*Power 3.1.[6] Following values were considered for estimating sample size; Type I (α) error- 0.05, power of the study 0.8, clinically significant minimum expected difference between groups (d) - 1.3 (pH units) [based on the study done by Goodson J et al].[7] Sample size estimated was 12. Anticipating 25% drop out, final sample size was approximated to 15. A convenient sample of undergraduate female students aged between 20–23 years of Bapuji Dental College and Hospital, Davangere city who fulfilled the eligibility criteria and consented to participate in the study were randomly selected. Participants who were on medications for any systemic diseases that affected their salivary flow and those who were unable to comply with the study time schedules were excluded. Voluntary informed consent was obtained from study participants before study commencement after explaining them about the study details and intended harms through a participant information form.

Preparation of interventional solutions

Goodson et al in their study showed beneficial effects of stevia at 4.7% concentration.[7] Hence, it was decided to test 4.7% of sucrose and stevia solutions in the present study. 4.7% stevia solution was prepared by adding 7 grams of stevia powder. [Product name: Nature velvet stevia powder; manufacturer: Natures Velvet Life care, Hyderabad] to 150 ml of distilled water until it was completely dissolved. (Photograph 1) Later the solution was filtered using a sieve to get a final solution. 4.7% sucrose solution was prepared by adding 7 grams of table sugar to 150ml of distilled water and stirring it for 5 minutes until completely dissolved. The dose calculation for solutions was determined by Department of Pharmacognosy, Bapuji Pharmacy College, Davanagere.

Photograph 1:Stevia Solution

Randomization

Random sequences of numbers were generated with the help of computer assisted software, followed by random allocation of subjects (concealed randomization) to the interventional groups. Random allocation was done by a person not involved in the study who was handed over random numbers within sealed opaque envelopes. Once a participant, fulfilling the eligibility criteria consented to enter the trial, an envelope was opened and the participant was then offered the selected group intervention.

Interventional Groups

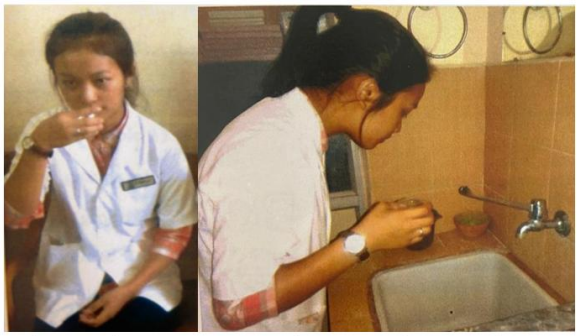
Group A (Test group)- Mouth rinsing with 30ml of 4.7% Stevia solution for 30 seconds.

Group B (Control group)- Mouth rinsing with 30ml of 4.7% Sucrose solution for 30 seconds.

Group C (Positive control)- Mouth rinsing with 30ml of distilled water for 30 seconds.

Intervention Details

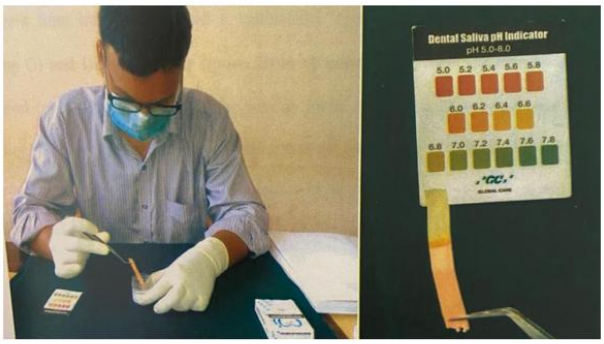
As it is a cross over Latin square design, all the participants were exposed to all the three interventions sequentially at different phases. Each group was subjected to all three interventions in a phased manner as shown in the schematic diagram (Figure 1). After random allocation, participant's baseline salivary pH was estimated. Then they were instructed to mouth rinse with test solutions for 30 seconds by swishing the entire content in the mouth at once and expectorate. (Photograph 2)



Photograph 2 :Participant mouth rinsing with stevia solution

Method of Saliva Collection: Participants refrained from eating for one hour before collection of saliva. Unstimulated saliva was collected by allowing the participants to pool the saliva in the floor of the mouth for at least 30 seconds and then expectorate into a sterile disposable cup. Around 2 ml of unstimulated saliva was collected.

Salivary pH estimation: Salivary pH indicator strips ((GC; Tokyo, Japan) were used to determine salivary pH with the help of reference provided by the manufacturer. Photograph 3)

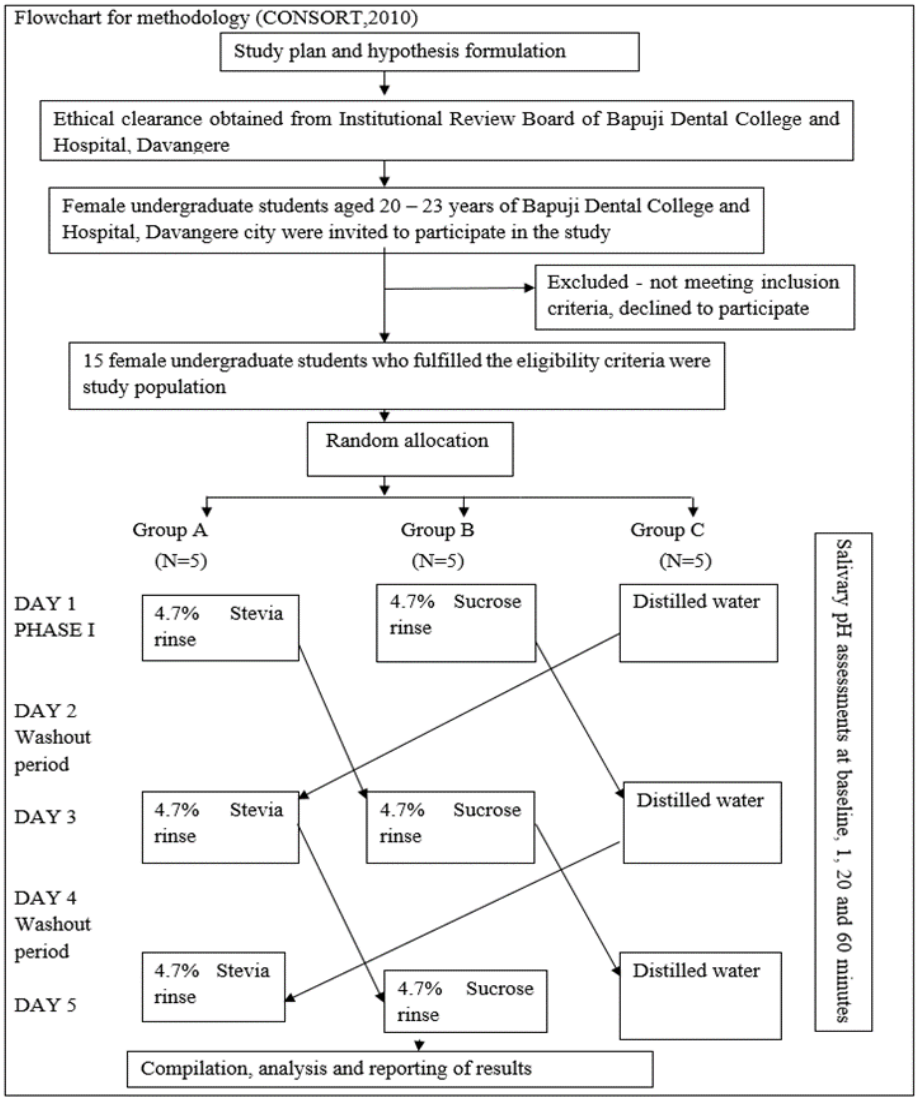


Photograph 3: Salivary pH estimation using pH indicator strips

Blinding: Investigator, participants and statistician were blinded to the interventional details of participants as the interventional solutions were put in a similar looking bottles, coded and then given to the participants by a person not involved in the study who allocated the participants to the group. The participant's group identifying details were also coded and later revealed after analysis.

Statistical Analysis: Data analysis was performed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Data was normally distributed with Shapiro-Wilk's test, so parametric tests were applied. Repeated measures and One way -ANOVA tests were employed to compare the means of salivary pH within the group at different time intervals and between the groups respectively. Post hoc Tukey's test was performed as significant difference was found between the groups and within the groups at different time intervals.

Figure 1: Schematic Representation of Methodology



RESULTS

There was a significant ($p<0.05$) increase in salivary pH (≥7) compared to baseline value (6.8±0.22) at various time intervals in the stevia group compared to sucrose group wherein, there was decrease in salivary pH (≤6.7) at all time periods compared to baseline (6.8±0.24). This decrease in pH, was significant ($p<0.05$) at 20 minutes and 60minutes. In distilled water group, there was no significant difference in salivary pH at all time periods compared to baseline pH (6.8±0.28) (Figure 2, Table 1). There were no adverse outcomes or unintended effects.

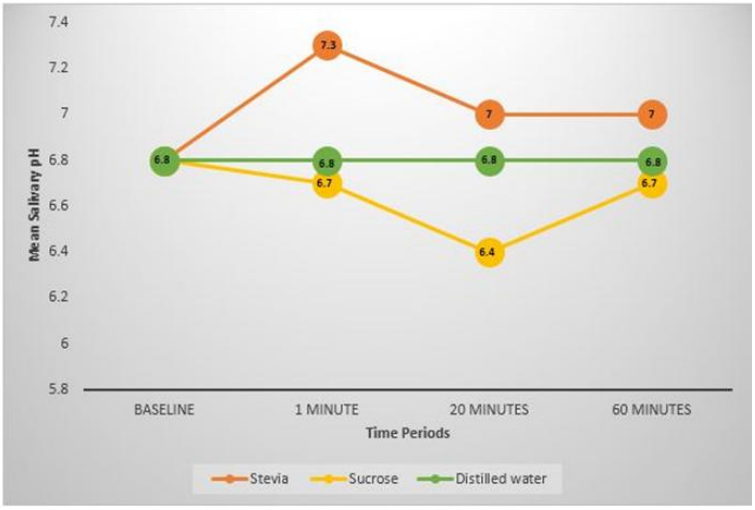
Table 1: Inter and Intra Group Comparison of Salivary pH at Various Time Intervals

Time	pH of Saliva (mean±SD)			One-way ANOVA Value(F), P value
	4.7% Stevia rinse (Group A)	4.7% Sucrose rinse (Group B)	Distilled water rinse (Group C)	
Baseline	6.8±0.22^{abc}	6.8±0.24^{fg}	6.8±0.28	F=0.6, P= 0.5
1 minute	7.3±0.19^{ABade}	6.7±0.29^{Ah}	6.8±0.23^{B}	F=16.3, P=0.001*
20 minutes	7.0±0.27^{CDbd}	6.4±0.29^{CEfhi}	6.8±0.25^{DE}	F=13.1,P=0.001**
60 minutes	7.04±0.32^{Fce}	6.7±0.22^{Fgi}	6.8±0.19	F=4.4, P=0.01*
Repeated measures ANOVA value, P Value	F=5.4,P=0.003	F=5.5,P=0.001	F=1.5,P=0.2	

*Statistically significant at p <0.05 and **statistically highly significant at p < 0.01.

Similar capital letter alphabets signify significant difference between groups (p<0.01) and similar small letter alphabets signify significant difference (p<0.01) within groups as determined by post hoc Tukey's test.

Figure 2: Line graph showing trends in salivary pH changes across groups



DISCUSSION

The results indicated that there was a significant difference in the effect of 4.7% Stevia solution, 4.7% sucrose solution and distilled water mouth rinsing on salivary pH. These results are in accordance with results of few studies where plaque pH was significantly higher after rinsing with stevia solution compared to sucrose solution rinsing.[7-11] Rinsing with 4.7% stevia solution showed rise in plaque pH than baseline pH at various time intervals (1 minute, 20 minutes and 60 minutes). Perhaps, the rise in salivary pH may be attributed to the alkaline nature of stevia.[12] Brambilla *et al*., investigated the effect of the Stevia extracts on plaque pH and reported that stevia did not support acidogenic metabolism from supragingival plaque bacteria. This could be due to the inhibitory effect of the Stevia on fermentative metabolism of bacteria. This study confirmed the cariostatic activity of the Stevia extracts by the suppression of bacterial growth in an invitro model.[13] Evidence shows that Octa-acetylombuoside, ombuine and retusine components of stevia exhibit anti-microbial action against few types of gram positive bacteria.[4]

According to Paraskevas *et al*., 30 second mouth rinsing was sufficient enough for all the tooth surfaces to come in contact with the rinsing solution.[14] Hence it was decided to allow 30s for mouth rinsing with interventional solutions. Unstimulated saliva collection was followed in the study as it does not show considerable variations in salivary pH.[15] Salivary pH changes was assessed at different time intervals (1 minute, 20 minute and 60 minutes) based on the concept of Stephen's curve which describes the changes in dental plaque pH in response to a carbohydrate challenge over a period of time. This curve demonstrates that, after the consumption of sugar there is a rapid drop in plaque pH and it normally takes at least 20 minutes to reach its resting value. Study by Azrak et al fixed similar time periods to assess the course of changes in salivary pH-values after intake of different beverages in young children.[16] A convenient sample of 20-23 years old female undergraduate students of same college who were residing in the common hostel were included in the study because their dietary patterns were similar thereby this study minimized the confounding effect of

diet, age and sex variations on salivary pH to a possible extent. Latin square design was employed to avoid subject variations within three different interventional groups.

The limitation of the present study was its small sample size which may affect the validity and generalizability of the study results. Estimation of plaque pH changes would be more specific and sensitive to carcinogenicity determination than salivary pH which is altered by various biologic and salivary factors. Hence, further trials involving large sample based on estimation of plaque pH changes after stevia use are recommended.

CONCLUSION

There was a gradual rise in salivary pH from baseline level till 1 hour after rinsing with stevia solution. Hence, the ability of stevia to maintain alkalinity of salivary pH along with its other anti-cariogenic properties provides scope to explore its utility as an anti-cariogenic product in various oral hygiene products. Stevia sugar may offer several advantages over other non-caloric sucrose substitutes since it is heat-stable, and non-fermentable sugar.

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Cite this article as:
Puja C Yavagal, Bhawana R Kumar, Divyapriya GK. Effect of 4.7% Stevia Solution Mouth Rinsing on Salivary pH: An In-Vivo Randomized Controlled Trial. International Journal of Ayurveda and Pharma Research. 2022;10(2):17-21.
https://doi.org/10.47070/ijapr.v10i2.2255
Source of support: Nil, Conflict of interest: None Declared

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