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Research Article

Stevioside Hydrate Effect on Growth, Acidogenicity and Adhesion of *Streptococcus Mutans In Vitro*

Anat Baniel[1]*, Sarit Faibis[1], Doron Steinberg[2] Nili Tickotsky[3] and Moti Moskovitz[1]

[1]*Department of Pediatric Dentistry, Hebrew University, Israel*

[2]*Institute of Dental Sciences, Hebrew University, Israel*

[3]*Bar Ilan University, Israel*

***Corresponding Author:** Anat Baniel, Department of Pediatric Dentistry, Hebrew University, Hadassah School of Dental Medicine, Jerusalem, Israel.

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Abstract

Background and aim: The present study examined the *in vitro* effect of pure glycoside Stevioside hydrate on pH, growth and biofilm formation of *Streptococcus mutans.*

Method: *S. mutans* were supplemented with different concentrations of Stevioside hydrate. Bacterial growth, metabolism and effect on biofilm formation were examined.

Results: The presence of Stevioside hydrate had no significant effect on: 1) *S. mutans* growth; 2) Total bacterial growth; 3) *S. mutans*' acidogenicity; 4) *S. mutans* adhesion in biofilm formation.

Conclusion: Stevioside hydrate has no cariogenic potential as demonstrated by acidogenicity, *S. mutans* growth and biofilm effect.

Keywords: *Stevioside hydrate; Streptococcus mutans; Biofilm; acidogenicity*

Introduction

The effect of various sweeteners on oral health and caries formation is well known. Following the establishment of the negative effect of sucrose on oral health and caries formation, most studies have focused on the effect of non-cariogenic sugar substitutes such as Sorbitol and Xylitol [1]. Various studies conducted around the world (mostly in children) demonstrated a 30-60% decline in caries formation following the use of chewing gum and dentifrice containing these sugar substitutes [2-8]. Xylitol has been demonstrated to be an effective sugar substitute, and the use of Xylitol or Sorbitol (or a combination of the two) as sucrose substitutes was proven to substantially lower caries rate [1].

Stevia is a calorie-reduced sweetener derived from the South American plant Stevia rebaudiana. The Steviol glycosides Stevioside and Rebaudioside A are the major components of Stevia and are the main contributors to its extremely sweet taste. The two basic forms of the Stevia plant, Stevioside or Stevia extract, are commonly used as sugar substitutes and as components of dentifrices and mouth rinses in South America and the Far East (Korea, Thailand, China and Japan). Recently there has been increasing interest in the use of this plant extract as a sugar substitute, due to its low caloric content which may reduce common effects of sucrose consumption such as obesity and diabetes mellitus. Moreover, this natural herb has fewer potential side effects than synthetic sweeteners.

Stevia-based sweeteners are approved for consumption as food additives in Brazil, South Korea and Japan. However, Stevioside and its derivatives are not yet considered Generally Recognized as Safe (GRAS) by the FDA, and are therefore categorized as dietary supplements

in the US as well as in the EU. In 2006 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) temporarily approved its Acceptable Daily Intake (ADI), which is currently up to 5.0 mg per kg of body mass.

According to the JECFA tests in 1999 no carcinogenic effects were found for an intake of no more than 2g per kg of body mass in men and 2.4g per kg of body mass in women [9]. Most studies agree that a reasonable daily intake of Stevioside (5 mg per kg of body mass) is safe and is neither carcinogenic nor teratogenic [10-13].

Dental caries is a significant health problem in many countries. Cariogenic bacteria produce acid due to fermentation of simple diet carbohydrates as sucrose. *Mutans streptococci* (MS) is one of the leading pathogens responsible for human caries. Prevention of dental caries would greatly benefit from the eradication of MS from the oral flora, an impairment of its ability to adhere to dental surfaces, or a reduction of its acidogenicity. In accordance with these goals, the aim of the present study was to determine the effects of various concentrations of Stevioside hydrate extracts (the pure extract of Stevia) on the pH, growth and biofilm formation of *Mutans streptococci in vitro.*

Materials and Methods

All experiments were conducted on *S. mutans* 27351 ATCC grown overnight in brain heart infusion (BHI) broth (Difco Laboratories, Maryland, USA) at 37°C in air atmosphere supplemented with 5% CO_2 (v/v). The bacteria were grown in BHI supplemented with different concentration of Stevioside hydrate, (Sigma, St. Louis, MO, USA) with or without 2% Sucrose. After an overnight incubation, rate of growth of bacteria was determined by light absorbance at 450 nm (Spectrophotometer, Camspec M302, Cambridge, UK).

Stevia's effect on bacterial metabolism was estimated through measurement of changes in media pH. Measurements were performed following incubation, using pH level indicator test strip (pH indicator strips, Mark, KGaA, Darmstadt, Germany).

Evaluation of the effect of Stevia on biofilm formation was performed using the crystal violet technique [14]. After incubation the growth media was removed and the biofilm was rinsed carefully with 1.0 ml of PBS. Biofilm on the tube walls was stained using 1% crystal violet. After 15 minutes the stained biofilm was rinsed thoroughly with PBS. The dye was then extracted using 70% ethanol, and the intensity of the dye absorbed to the biofilm was determined using a 595 nm Genius spectrophotometer (Genius, Tecan, Männedorf, Switzerland)

Data was analyzed using SPSS software version 20. The analysis consisted of Kruskal-Wallis non parametric tests. Significance level was set at $p \leq 0.05$.

Results

Kruskal-Wallis test showed no differences between *S. mutans* growth rate on the various Stevioside hydrate concentrations as compared to the control ($P = 0.12$) (Figure 1). Total bacterial growth with 2% sucrose addition to the media was also not affected by the presence of Stevioside hydrate ($P = 0.08$) (Figure 2). Stevioside hydrate had no significant effect on *S. mutans* adhesion during biofilm formation ($P = 0.12$) (Figure 3). *S. mutans* acidogenicity was not influenced by the presence of Stevioside hydrate at the tested concentrations, with the pH remaining identical throughout the different concentrations.

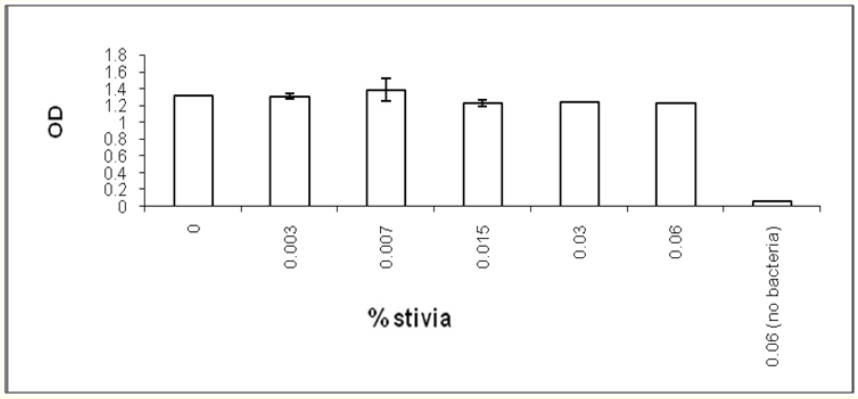

Figure 1: *The effect of Stevioside hydrate on bacterial growth. OD on the Y axis signifies Optical Density.*

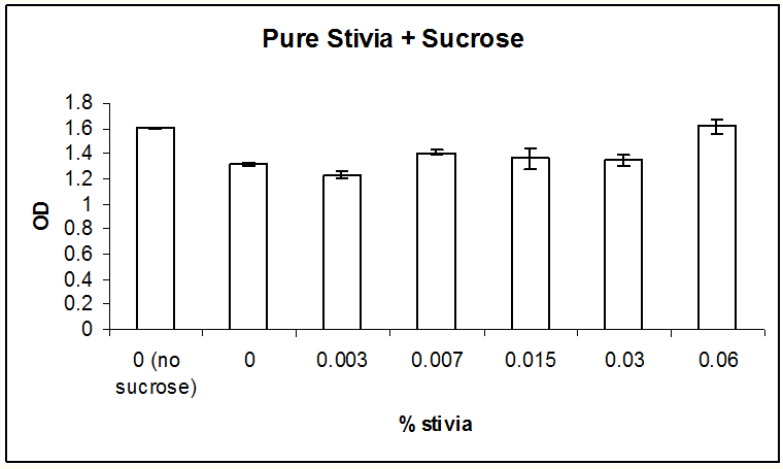

Figure 2: *The effect of different concentrations of Stevioside hydrate and 2% sucrose on bacterial growth in BHI. OD on the Y axis signifies Optical Density.*

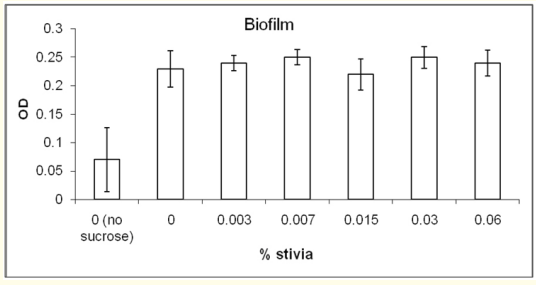

Figure 3: *Biofilm crystal violet stain- Crystal violet stain of S. Mutans biofilm in the presence of different concentrations of Stevioside hydrate and 2% sucrose. OD on the Y axis signifies Optical Density.*

Discussion

Stevia is a natural non sugar substitute used as calorie free ingredient. Few studies have explored its effect on oral bacteria and most of them had used Stevia extracts [15,16] rather than the pure compound of Stevioside hydrate. The present study's distinctiveness is in the use of pure Stevioside hydrate. The decision to use pure Stevioside hydrate was based on the concept that only the use of purified Stevioside hydrate, Steviol or Rebaudioside A may enable significant progress in the investigation of those ingredients' affect on the oral flora.

The present study demonstrated that Stevioside hydrate did not influence *S. mutans*' growth: it neither promoted *S. mutans*' growth nor inhibited it. Stevioside hydrate also did not influence the acidogenicity of those bacteria, as the pH values after bacterial incubation did not change with or without Stevioside hydrate. In addition, Stevioside hydrate did not induce nor inhibit biofilm formation.

The present outcomes support those obtained by Zanela., *et al*. [15], who found that Stevia and fluoride mouth rinses had no anti-biofilm effect, and also no effect on *S. mutans* and *S. sobrinus* salivary levels. Das., *et al*. [16] found that 0.5% Stevioside and 0.5% Rebaudioside A had no cariogenic effect and no effect on *S. sobrinus* counts in rats. Mouth rinses containing Rebaudioside A were not found more acidogenic than water or sucralose in humans [17]. The results of the present study are in accordance with those studies.

The results obtained in this *in vitro* study indicate that Stevioside hydrate, the active glycoside of Stevia extract, has no cariogenic potential as depicted by acidogenicity, *S. mutans*' growth and biofilm effect. Unlike Xylitol, Stevioside hydrate has no adverse cariogenic effect such as bacterial growth or acid generation due to fermentation. In addition, it has no positive or negative effect on biofilm formation. Further studies with pure Stevioside hydrate are necessary to determine the effect of this substance on other cariogenic bacteria.

Conclusion

Stevioside hydrate has no cariogenic potential as demonstrated by acidogenicity and no effect on *S. mutans* growth and biofilm formation.

Bullet Points

The sweetener Stevioside hydrate has no cariogenic potential as demonstrated by acidogenicity and no effect on *S. mutans* growth and biofilm formations.

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