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ORIGINAL RESEARCH

The Antimicrobial Activity of the Three Commercially Available Intense Sweeteners against Common Periodontal Pathogens: An *in vitro* Study

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ABSTRACT

Aim: To evaluate the *in vitro* antimicrobial activity of three commercially available intense sweeteners against two common periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

Materials and methods: Three commercially available intense sweeteners namely saccharin, aspartame and sucralose were obtained and powdered. Necessary concentrations of the sweeteners were prepared by mixing them with an inert solvent. The antimicrobial efficacy was assessed using agar well diffusion technique. Statistical analysis was done using one-way ANOVA followed by Tukey's post hoc test. p-value < 0.05 was considered statistically significant.

Results: All the three sweeteners showed significant antimicrobial activity against the periodontal pathogens tested. Sucralose containing sucralose showed maximum zone of inhibition, against *Aggregatibacter actinomycetemcomitans.* Saccharin and aspartame containing saccharin and aspartame respectively, showed maximum zone of inhibition, against *Porphyromonas gingivalis.*

Conclusion: All the sweeteners used in this study have demonstrated significant antimicrobial activity. Therefore, these sweeteners could be recommended as an ideal alternative to sucrose.

Clinical significance: Dental caries and periodontal diseases are ubiquitous diseases of mankind caused by microorganisms. Dental caries is caused by sucrose. By altering the source like intense sweetener we can combat caries as well as with its antimicrobial properties against periodontopathic bacteria, we can reduce prevalence of periodontal diseases.

Keywords: Intense sweeteners, *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans,* Antimicrobial activity, Saccharin, Sucralose, Aspartame, *In vitro* study.

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INTRODUCTION

Human beings have inborn liking sensation of sweetness. Indulgence in sweets has been described as 'universal human weakness'. Sweetness is the taste that is strongly identified with affection and reward. Sucrose (common sugar), originally obtained from sugarcane, has remained the primary sweetening agent since 17th century.[1]

From an oral health perspective, increased sugar consumption has been associated with dental caries,[2,3] periodontal disease[4] and oral candidiasis.[5]

The pathogenesis of periodontal disease is complex because it reflects a combination of the initiation and maintenance of the chronic inflammatory process by a diverse microbial flora and its numerous bacterial products. The subsequent host response to this infection mediates complex cascade of tissue-destructive pathways. Additional factors contributing to this multifaceted local disease process in the oral cavity include a number of systemic diseases, especially diabetes, that can exaggerate the host response to the local microbial factors (for example, endotoxin), resulting in unusually destructive periodontal breakdown.[6]

Although diabetes mellitus has long been considered a risk factor for the development of periodontal disease, however, there is also evidence that periodontal disease can worsen a patient's control of diabetes mellitus and that proper management of periodontal disease can improve control of diabetes mellitus.[7]

The increased prevalence of these diseases, such as diabetes mellitus, coupled with an increasing diet conscious society has led to the growth of artificial sweeteners in the market.[8] Artificial sweeteners are either bulk sweeteners or intense sweeteners. Artificial or natural substances with many times the sweetening power of sucrose, but no or negligible calorific value, are known as intense sweeteners.[8]

These sweeteners are widely used in the manufacture of diet foods and beverages, food for diabetic patients, tooth friendly products, etc.[8] From an oral health perspective, few studies have demonstrated antibacterial activity of few intense sweeteners against some oral pathogens[9] but the studies on the antibacterial properties of the sweeteners against the periodontal pathogens are rare.

According to the Consensus report of the World Workshop on Clinical Periodontics (1996), human periodontitis is initiated and perpetuated by a small group of bacteria that colonize the subgingival region, mainly Gram-negative, anaerobic or microaerophilic bacteria. Furthermore, most cases of human periodontitis are caused by *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans.*[10,11]

MATERIALS AND METHODS

The materials used in this study were:

1. Commercially available intense sweeteners:
   a. Saccharin
   b. Aspartame
   c. Sucralose
2. Microorganisms
   a. *A. actimomycetemcomitans* ATCC 43718
   b. *P. gingivalis* ATCC 33277
3. Blood agar with hemin and vitamin K supplements
4. Vernier calipers.

Three commercially available intense sweeteners with active ingredients saccharin, aspartame and sucralose were obtained from a local medical store.

Necessary concentrations of the sweeteners were prepared by mixing the appropriate amount of the sweeteners with an inert solvent dimethyl sulfoxide (negative control) and used in the analysis.

Agar Well Diffusion Assay (Perez et al 1990)[12]

Agar well diffusion assay was the key process used to evaluate the antimicrobial potential of the extracts. Petri dishes containing 18 ml of blood agar supplemented with vitamin K and Hemin were inoculated with approximately 100ìl of *A. actimomycetemcomitans* and *P. gingivalis* strain using the swab technique.

Wells of 8 mm diameter were cut into the solidified agar media using a sterilized standard device. A total of 100 µl of the each of the test solutions were poured in the well and the plates were incubated at 37°C for 48 hours. To ensure the consistency of all the findings, the experiment was performed and repeated under strict aseptic conditions. The antimicrobial activity of the solutions were expressed in terms of the mean diameters of zone of inhibition (in mm) produced at the end of the incubation period.

STATISTICAL ANALYSIS

The mean and standard deviation of the diameter of inhibition zone was calculated. Statistical significance were measured by using one-way ANOVA followed by Tukey's post hoc test. The p-value < 0.05 was considered statistically significant. The statistical analysis was done using the Statistical Package for Social Sciences (SPSS) software, version 17.0.

RESULTS

Table 1 shows the antimicrobial activity of the sweeteners on *A. actinomycetemcomitans* as determined by agar well diffusion method on specific media. All the three sweeteners showed significant antimicrobial activity against the organism. Of the three sweeteners, sucralose containing sucralose showed maximum zone of inhibition, followed by aspartame containing aspartame and Saccharin containing saccharin.

Table 2 shows the antimicrobial activity of the sweeteners on *P. gingivalis* as determined by agar well diffusion method on specific media. All the three sweeteners showed significant antimicrobial activity against the organism. Of the three sweeteners, aspartame containing aspartame and Saccharin containing saccharin showed maximum zone of inhibition, followed by sucralose containing sucralose.

DISCUSSION

The present study evaluated the *in vitro* antimicrobial efficacy of three commercially available intense sweeteners against *P. gingivalis and A. actinomycetemcomitans.* These are among the most commonly used and marketed intense sweeteners in India.[13]All the three intense sweeteners used in the study have been approved for use by the ADA/ USFDA in 2004.[14]

The products used in this study have demonstrated anticariogenic/noncariogenic properties in earlier studies. However, no study has been reported in the available electronic literature that determines the antimicrobial activity of these sweeteners against *P. gingivalis and A. actinomycetemcomitans.*

All the sweeteners, in addition to the active ingredients, contain binding agents, such as magnesium stearate and polyvinyl pyrollidone. However, no antimicrobial activity of these agents has been demonstrated. Hence, the observed antimicrobial activity may be attributed to the active ingredients.

Table 1: Antimicrobial activity of the sweeteners on *A. actinomycetemcomitans* as determined by agar well diffusion method on specific media

Sl. no.	Sweeteners	Mean zone of inhibition (mm)	Standard deviation	ANOVA	Tukey's post hoc
1.	Saccharin (Sweetex®)	5.87	0.28	F-value =	3>2>1>4
2.	Aspartame (Sugar Free Gold®)	18.87	0.30	29726.738	
3.	Sucralose (Sugar Free Natura®)	37.99	0.30	p < 0.001	
4.	Dimethyl sulfoxide (negative control)	0	0	(significant)	

Table 2: Antimicrobial activity of the sweeteners on *P. gingivalis* as determined by agar well diffusion method on specific media

Sl. no.	Sweeteners	Mean zone of inhibition (mm)	Standard deviation	ANOVA	Tukey's post hoc
1.	Saccharin (Sweetex®)	38.2	0.33	F-value =	2 = 3>1>4
2.	Aspartame (Sugar Free Gold®)	45.9	0.26	4893.181	
3.	Sucralose (Sugar Free Natura®)	52.0	0.33	p < 0.001	
4.	Dimethyl sulfoxide (negative control)	0	0	(significant)	

Against *A. actinomycetemcomitans*, sucralose containing sucralose showed maximum inhibition followed by aspartame and saccharin and against *P. gingivalis*, aspartame and saccharin showed maximum inhibition followed by sucralose.®

Sucralose is manufactured by selective chlorination of sucrose followed by acetylation.[14] The antimicrobial activity demonstrated in this study may be attributed to the presence of chlorine.

Aspartame is made up of aspartame, which is a dipeptide of phenylalanine and aspartic acid linked to methanol (hence contraindicated in patients with phenylketonuria). The observed antimicrobial activity may be attributed to the presence of aspartic acid which has proved antifungal activity.[15]

Saccharin has demonstrated antibacterial activity against intestinal pathogens[10] and *Streptococcus mutans*. This activity is attributed to the inhibition of the glucose transport mechanism in these organisms.[12]

Although these products have shown antimicrobial activity, further studies need to be conducted to ascertain the exact mechanism of antimicrobial activity and minimal inhibitory concentration. With proven safety on humans, these products can be incorporated into various oral care products, beverages and confectionaries, thereby aiding in *in vivo* clinical trials.

CONCLUSION

This study evaluates the antimicrobial effect of some commercially available intense sweeteners against *P. gingivalis* and *A. actinomycetemcomitans*. All the sweeteners used in this study have demonstrated significant antimicrobial activity against the two common periodontal pathogens. With the proved antibacterial, anti/non-cariogenic properties and safety, these sweeteners could be recommended as an ideal alternative to sucrose.

CLINICAL SIGNIFICANCE

Dental caries and periodontal diseases are ubiquitous diseases of mankind caused by microorganisms. Dental caries is caused by sucrose. By altering the source like intense sweetener we can combat caries as well as with its antimicrobial properties against periodontopathic bacteria, we can reduce prevalence of periodontal diseases.

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