COMPARATIVE EVALUATION OF ANTI CARIOGENIC POTENTIAL OF SUGAR SUBSTITUTES ON S. MUTANS AMONG CHILDREN BETWEEN THE AGE GROUP OF 9-12 YEARS: AN IN-VIVO STUDY

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ABSTRACT

Background: Colonization of teeth by Streptococcus mutans is one of the most important risk factors in the development of dental diseases. The dental plaque is a structure of vital significance as a contributing factor to at least the initiation of the carious lesion. S. mutans is considered to be the chief etiological agent in human dental caries. The reduction of sugar intake and its replacement with non-fermentable sweeteners is considered a useful approach to caries prevention. These sugar substitutes show anti-bacterial and anti-cariogenic activity at a stipulated quantity. Aim: The aim of the present study was to evaluate and compare the in-vivo antimicrobial efficacy of sugar substitutes, Xylitol, stevia and honey against S.mutans in plaque in children between the age group of 9 to 12 years. Methodology: For the purpose of the study, a total of 60 children were selected and randomly divided into 3 groups of 20 each. First the plaque samples were taken at a baseline score zero. Then the children were given the prepared solutions of the sugar substitutes and instructed to swish in the mouth for 1 minute before swallowing it or spitting. Plaque samples were taken again after 1 hour and was taken to the laboratory for microbial analysis. Microbiological analysis was done using selective media, Mitis Salivarius Bacitracin (MSB) Agar. Results: Statistical analysis using paired t-test & Tukey post hoc analysis showed that there was a significant reduction in the plaque S. mutans count from baseline to post rinsing in all the groups (p = 0.000) among which Xylitol has shown to have the highest antimicrobial action followed by stevia and then followed by honey. Conclusion: Thus, concluding that sugar substitutes are highly effective in reducing plaque S. mutans count in children.

Keywords: Sugar Substitutes, Xylitol, Stevia, Honey, S. mutans, Dental Caries

INTRODUCTION

Dental caries is the destruction of enamel, dentin, or cementum of teeth due to bacterial activities, which if left untreated can cause considerable pain, discomfort, and treatment costs are very high. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by cariogenic bacteria.[1] The dental plaque is a structure of vital significance as a contributing factor to at least the initiation of the carious lesion. S. mutans is considered to be the chief etiological agent in human dental caries. [2] The reduction of sugar intake and its replacement with non-fermentable sweeteners is considered a useful approach to caries prevention. Renewed interest in developing an

antimicrobial approach for the management of dental caries has evolved as a result of: Identifying certain members of the oral microflora as major cariogens and increased understanding of the specific ecology of these cariogens. In conjunction with this concept, control and prevention of caries has been sought by reducing the number of colonizing bacteria. Reducing their level in the oral cavity will provide an additional rationale for the prevention of dental caries.[3]The role of sucrose and other fermentable carbohydrates in the etiology of dental caries has been well established. Since it is known that sugared substances may increase the risk of dental caries, it has been proposed that the replacement of sucrose with a sugar substitute, may contribute to caries prevention. Sugar substitutes can play an important role in shifting the caries process in favor of maintaining dental health and they should be recommended as part of an overall preventive treatment plan for patients at high risk of developing caries.

Xylitol, a naturally occurring five-carbon sugar polyol, is a white crystalline carbohydrate known since a century ago. [4] It has been widely researched and globally accepted as a natural sweetener approved by the US Food and Drug Administration (FDA) and the American Academy of Pediatric Dentistry. [5]

Stevia is a noncaloric sweetener derived from Stevia Rebaudiana plant species, used in patients with diabetes and hypertension. [6,7] It is a subject of dental research as it is a natural substance which treats a variety of ailments with its antibacterial and antifungal properties.[8]The antibacterial properties of honey have been well documented.[9] However, the specific antimicrobial mechanism of honey is still unclear. [10] Among the possible mechanisms are the presence of inhibitory factors such as flavonoids [11] and hydrogen peroxide, [12, 13] low pH, and high osmolarity due to its sugar concentration. [14]Mothers often give children sugar containing food or sugar-coated chocolates as snacks or after any spicy food and whenever they demand with no heed of rinsing off their mouths after eating. This increases the risk of caries by double fold by decreasing plaque pH, metabolizing the sugar and resulting in demineralization. Controlling the frequency of intake of dietary sugar and use of alternative sugars in foods have therefore been recommended as the preventive strategy for dental caries management. [15] Sugar substitutes are those that cannot be metabolised by the cariogenic organisms, thus cannot lower the biofilm pH on the tooth surface.Hence, the aim of the present study was to evaluate the anticariogenic potential of sugar substitutes on Streptococcus mutans among children between the age group of 9-12 years.

MATERIALS AND METHODS

Prior to carrying out this study, ethical approval was obtained from the institutional ethics panel, and proper written consent was acquired from each parent or guardian in the language understood by them. For the purpose of this study 60 children aging between 9-12 years was selected. Children were randomly divided into three groups, 20 in each group.The inclusion criteria were children between the age group of 9-12 years, Children with decayed teeth with DMFT Score >3, Children with early-stage enamel decay according to the International Caries Detection and Assessment System (ICDAS) II criteria [16] and Children who were not on any long-term medication prior to or during the study period. Children with DMFT/dmft >3 were included because a higher DMFT results in lower pH levels as compared to no DMFT.[17] Any subjects with special healthcare needs, systemic diseases, current or recent use of antibiotics and undergoing any dental treatment, orthodontic treatment were excluded from this study.

CLINICAL TRIAL

At the start of the clinical trial, the subjects were instructed not at eat or drink anything 2 hours prior to the procedure. The study sample was divided randomly into 3 groups i.e.

Group 1: Xylitol [So Sweet Xylitol, Herb Veda]

Group 2: Stevia [Premium liquid Stevia, Bliss of Earth] Group 3: Honey [Patanjali Honey]

Aqueous solution of Xylitol was prepared by adding 8gms of xylitol to 100ml sterilized distilled water (8%) [18]

Aqueous solution of Stevia was prepared with 25ml of Stevia in 100ml of sterilized distilled water (25%) [19]

Aqueous solution of honey was prepared by adding 21ml of honey in 100ml of sterilized distilled water (21%) [9]

The study commenced by collecting plaque samples with the help of dental explorer from labial aspect of deciduous primary incisors/ buccal aspect of maxillary deciduous molars and lingual aspect of mandibular deciduous incisors and molars as the baseline score. Collection of plaque samples was done from the buccal and lingual surface of 16, 11, 64, 36, 41, and 84. These plaque samples were transferred to an eppendorf containing 1ml of normal saline solution. The children were then randomly divided into 3 groups and given the solutions and asked to swish the mouth with 5ml of the respective solutions of xylitol, stevia and honey for 1 minute. The children were asked to either swallow the solutions or spit it according to their will. Plaque samples were collected once again after 1hour and the samples were then taken to the laboratory for microbial analysis. All the samples were collected in aseptic environment. The microbial samples were collected in eppendorf containing 1ml of normal saline and carefully the lid of the eppendorf was closed tightly.

LABORATORY PROCEDURES

The microbial analysis was carried out in the Biotechnology department in the Guru Ghasidas University, Bilaspur. In the laboratory, the samples were vortexed for 15 seconds to dislodge the microorganisms. The plaque samples were serially diluted to 1:10,000 times (104). Pipetting of 100µL volume of each sample into each agar plate containing the prepared selective media. Spread plate method was used to disperse the samples, so L- shaped spreader was used to spread the samples uniformly on the prepared selective media. Samples were inoculated in selective media - Mitis Salivarius Bacitracin Agar (MSB) - for Streptococcus mutans. The petri plates were incubated at 37°C. Streptococcus mutans levels were assessed after 36-72 hours of incubation under anaerobic conditions. Gram staining was done to confirm the presence of S. mutans. Appeared colonies were observed according to their morphological characteristics and finally, mean colony forming units (CFU/ml) of Streptococcus mutans were calculated from plates with > 10 and < 100 using digital colony counter (Figure 1, Figure 2). Computation of the number of colonies was done by multiplying the obtained count with 1×104 as the sample was diluted ten thousand times.

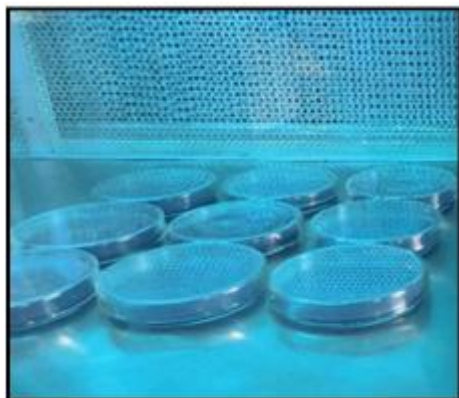*Eur. Chem. Bull.* **2023**,12(issue 8), 6478-6488

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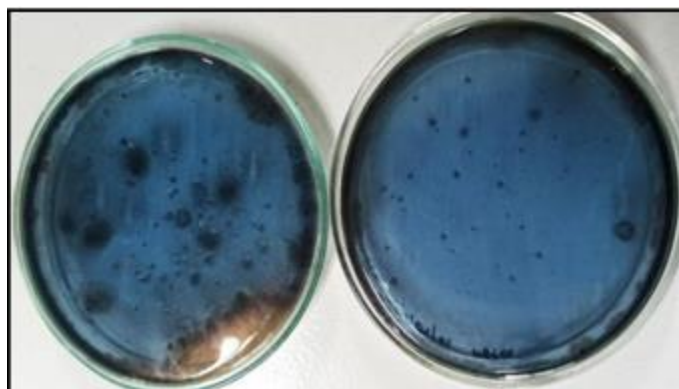Figure 1: inoculation of samples in the Laminar Air Flow

Figure 2: Before and After Growth of bacteria after incubation of 48 hours

STATISTICAL ANALYSIS

Data was analysed using paired-t test and Tukey Post Hoc Analysis in SPSS V.24 software. The level of significance was taken at P≤ 0.05. inter group comparison of the groups were done by Paired t-test and the intra-group comparison was done by Tukey Post Hoc Analysis.

RESULTS AND OBSERVATIONS

At the end of 48 hours, statistically significant antimicrobial activity was demonstrated by all the test specimens used in this study (P = 0.000). The antimicrobial activity of the extracts against S. mutans at 48 hours are as follows.

Groups	Before (x104)	After (x104)	Mean change	t value	p value
Xylitol	76.5±1.9	21.7±2.8	54.8	54.9	0.000*
Stevia	75.7±2.9	26.6±1.4	49.1	47.3	0.000*
Honey	75.3±3.6	38.9±2.1	34.4	30.7	0.000*

*Statistically significant difference exists between the groups (p <0.05)

Table 1. Comparison between before and after microbial counts (x104) in plaque in the groups using paired t test. Compares the before and after mean Streptococcus mutans count in plaque using paired T-test. It shows that the mean difference between the before and after values in Xylitol was found to be the highest 54.8 followed by the Stevia group with mean difference of
49.1 followed by the Honey group with a mean difference of 34.4

Graph 1. Comparison between before and after microbial counts (x104) in plaque in the groups using paired t test. It depicts that the reduction of Streptococcus mutans count in the Xylitol group was the highest. This was followed by the stevia group which was followed by the Honey group. Honey showed the least reduction of Streptococcus mutans as compared to the other two groups.

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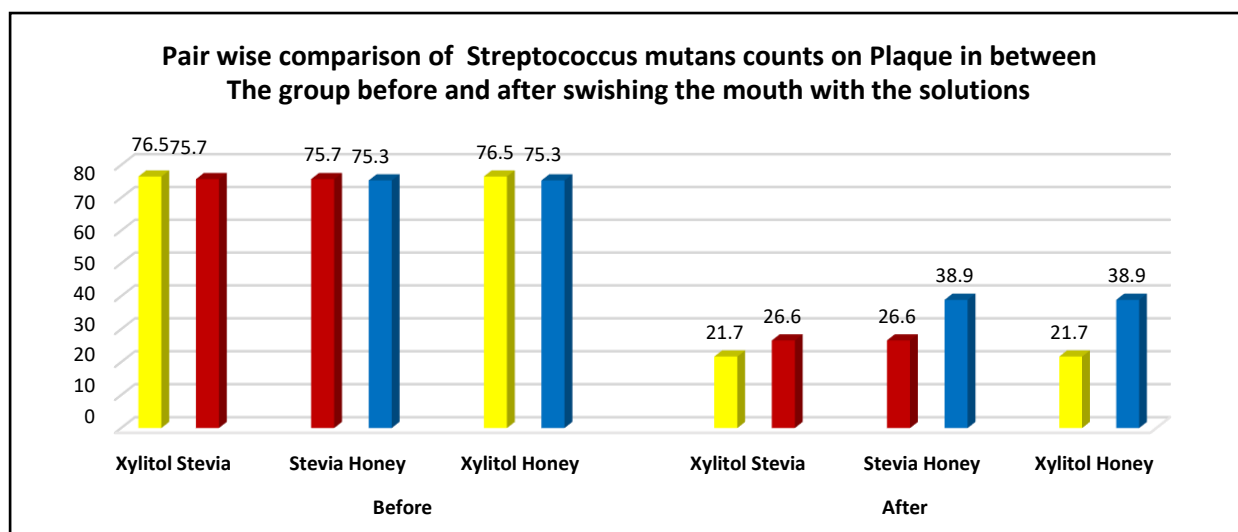
Graph 2: Pairwise comparison of before and after Streptococcus mutans counts (x104) in plaque in between the groups using Tukey post hoc analysis. This graph compares the mean Streptococcus count between the study groups after the use of the solutions of sugar substitutes. On comparing Stevia with Xylitol and Honey, a significant reduction of S. mutans count was seen by Stevia. On comparing Xylitol with stevia and honey, Xylitol had better action in reducing bacterial count. Among Stevia and Honey, Stevia showed more reduction of S. mutans count. While Honey has shown the least reduction of bacterial count in all comparisons.

DISCUSSION

Dental caries is one of the most common preventable diseases which is recognized as the

primary cause of oral pain and tooth loss. It is a major public health oral disease which hinders the acheivement and maintenance of oral health in all age groups. [20] WHO claimed that poor oral health may have a profound effect on general health as well as quality of life, and several oral diseases are related to chronic diseases. [21] Three steps are involved in the formation of dental plaque; First salivary molecules are adsorbed to the enamel as soon as a tooth has been cleaned. Hence the enamel is coated with a complex mixture of components that include glycoproteins, acidic proline-rich proteins, mucins, bacterial cell debris, exoproducts, and sialic acid. The second step is bacterial interaction with this acquired pellicle via several specific cell-to-surface interactions. [22]Mutans Streptococci (MS) colonization of an infant may occur from the time of birth. [23-28] Significant colonization occurs after dental eruption as teeth provide non-shedding surfaces for adherence. Other surfaces also may harbor MS. [29, 30] Vertical transmission of MS from mother to infant is well documented. [31-37]Sucrose has been implicated as an important determinant of dental caries disease. It serves as a substrate for synthesis of intracellular and extracellular polysaccharides in dental plaque [38] and is easily fermentable when compared to other starches. [39] To avoid the role of sucrose on the virulence factors of S. mutans, the use of sugar substitutes is considered. Sugar substitutes are those that cannot be metabolized by cariogenic micro-organisms thereby lead to lower or no production of acids, and are not substrates for glucan or fructan production and cannot lower biofilm pH thus reducing the pathogenic potential of dental plaque.Low-calorie sweeteners (sometimes referred to as non-nutritive sweeteners, artificial sweeteners, or sugar substitutes) are ingredients added to foods and beverages to provide sweetness without adding significant amount of calories. [40]Herbal interventions in dental caries management have been largely investigated. Herbal plant used in this study is Stevia Rebaudiana. Stevia rebaudiana Bertoni, which is a shrub of the Asteraceae family originating from the northeast part of Paraguay, is the source of noncaloric sweetening compounds, i. e. steviol glycosides. Stevia is approved as a food supplement in several countries such as Brazil, Japan, the United States and recently the European Union. [41] It has also been widely used for its antibacterial effect and is widely used in pharmaceutical industry. Inspite of its sweetness that is 300 times more than sugar,it is found to be not a cariogenic substance. As the extract of stevia has antibacterial and antifungal effect, it alsoraised an interest to find its efficacy against cariogenic organisms. Stevia is composed of reducing sugars (4.5%), moisture (10.73%), fibre (5.3%), proteins (13.68%), fat (6.13%) andcarbohydrates (63%).Stevia has been found to be effective in reducing the cariogenic microbial

count in the current study. Honey is super- saturated, delicious, and naturally sweet nectar popular worldwide and is collected by bees from a wide variety of plants. It contains carbohydrates which includes monosaccharides fructose (38.2%) and glucose (31%); and disaccharides (~9%) sucrose, maltose, isomaltose, maltulose, turanose and kojibiose and some oligosaccharides (4.2%), including erlose, theanderose and panose.In addition to these it contains proteins, aminoacids, vitamins, minerals, enzymes and antioxidants. [42] Honey has antibacterial activity against cariogenic bacteria such as S. mutans and Lactobacillus. [43] Factors that are effective in antimicrobial activity of honey include the osmotic effect, enzymatic glucose oxidation reaction, production of hydrogen peroxide, high osmotic pressure, a low pH, and the presence of phenolic acids, lysozyme, flavonoids, phytochemicals, antioxidants, beeswax, nectar, pollen, and propolis. [44] Honey contains factors that may reduce the solubility of exposed enamel in an acid buffer solution, compared to pure sucrose. In addition to the solubility- reducing substances, honey contains factors that may also reduce bacterial effects on dental caries. [45] Honey has three enzymes like diastase (amylase), decomposing starch or glycogen into smaller sugar units, Invertase (sucrose, α-glucosidase), decomposing sucrose into fructose and glucose, as well as glucose oxidase, producing hydrogen peroxide and gluconic acid from glucose. [46] This H_2O_2 produced is responsible for the antibacterial activity of honey.

In this study, honey has proven to have antimicrobial properties. The results show that stevia has better antimicrobial efficacy as compared to honey. In comparison to Xylitol, it was found that Xylitol had the highest antibacterial efficacy among the three groups that have been evaluated. (Table 1, Graph 1, Graph 2). It was found to be the most potent anti- bacterial agent, causing highest reduction of streptococcus mutans count.Xylitol is industrially produced from xylose (wood sugar), which is available in the forms of xylans (hemi cellulose polysaccharides that consist of xylose). Xylose is present especially in hardwood material, such as birch, beech, nutshells, straw, corn, and bagasse. Xylitol is a natural carbohydrate like substance. Xylitol is hydrophilic and may compete with water molecules for the hydration layers that surround protein molecules in biological environments. Furthermore, xylitol may form complexes with inorganic ions such as Ca^{2+}, stabilizing the calcium phosphates in saliva. [47] Marttinen AM et al in 2012 stated that Streptococcus mutans transports the sugar into the cell in an energy-consuming cycle that is responsible for growth inhibition. Xylitol is then converted to xylitol-5-phosphate via phosphoenolpyruvate: fructose phosphotransferase system by S. mutans resulting in development of intracellular vacuoles and cell membrane degradation. [48] Unwittingly contributing to its own death, S. mutans then dephosphorylates xylitol-5-phosphate. The dephosphorylated molecule is then expelled from the cell. This expulsion occurs at an energy cost with no energy gained from xylitol metabolism. Thus, xylitol inhibits S. mutans growth essentially by starving the bacteria. Xylitol can inhibit the growth of harmful oral bacteria such as S. mutans, but its benefits do not stop in the oral cavity. [49]Nordblad A et al (1995) said that Xylitol decreases the incidence of dental caries by increasing salivary flow and pH [50] and reducing the number of cariogenic (MS) and periodontopathic (Helicobacter pylori) bacteria, plaque levels, xerostomia, gingival inflammation, and erosion of teeth. [51] The results are in conjunction with the Turku sugar studies which were initiated in the beginning of 1970s. Scheie AA et al in 1998, did a prospective controlled, double-blind clinical trial which confirmed that Mutans Streptococci levels in plaque decreased as exposure to xylitol increased.

These are in conjugation with this study which shows that xylitol has the highest antimicrobial efficacy followed by Stevia and then followed by Honey.

CONCLUSION

Taken together, these results suggest that artificial and natural sweeteners have lower cariogenic potential with Xylitol having the highest antimicrobial efficacy followed by Stevia and lastly by Honey. Therefore, the anticariogenic potential of xylitol has shown to be the highest followed by Stevia followed by Honey. Further researches with longer duration needs to be done to check for the long term anticariogenic effects of sugar substitutes.

CONFLICTS OF INTEREST

There were no conflicts of interest.

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