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Comparative Evaluation of Cariogenic Potential of Natural and Unrefined Sweeteners on Streptococcus Mutans Biofilm Formation and Enamel Demineralization-In Vitro Study

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Abstract: *Dental caries is the most common infectious diseases found in human beings.Controlling the frequency of intake of dietary sugar and use of alternative sugars in foods have therefore been recommended as the preventive strategy for public and private in dental caries management. For biofilm assessment Six 10% solutions containing xylitol, sucrose, honey, jaggery, palmsugar and stevia were prepared. MTT assay was used to evaluate microbiological counts in vitro.For enamel demineralization assessment, a total of 120 extracted premolars were immersed in six group of sugar solutions and 1.5 × 108 cells of Streptococcus mutans were inoculated into each group for 21 days. Buccolingual sections of teeth were evaluated at three points under a polarized microscope. Higher in vitro S. mutans biofilm formation and was observed in sucrose solution ($p < 0.01$).Least invitro biofilm formation and depth of enamel demineralization was found for xylitol followed by stevia,honey,jaggery,palmsugar and the highest was with sucrose group. Within the limitations of the present study, it may be concluded that even though xylitol group exhibited the least mean depth of enamel demineralization, it cannot be statistically proved better than the stevia and honey group.*

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I. Introduction

Dental caries is a disease that is characterized by the localized destruction of susceptible dental hard tissue by acidic by-products from bacterial fermentation of dietary carbohydrates.[1] The significance of micro-organisms in the aetiology of dental caries has been highlighted in the ecological plaque hypothesis [2].Though Streptococcus mutans and Lactobacilli have been identified as the main cariogenic organisms, the Keyes' circle effectively describes the interaction of the causative factors in dental caries, namely, host, diet, microbes and time. A prolonged interplay of these factors result in loss of tooth structure in the form of a carious lesion.[3]

A recent article has reported a strong association between dietary sugars and dental caries. Despite the use of fluoride, 10% consumption of sugar has been found to increase the incidence of caries as per the WHO 2015 document [4]. Streptococci mutans metabolise the dietary sucrose and release an acidic by-product that results in demineralisation of the tooth. The organisms also produce extra-cellular polysaccharides (EPS) and intra-cellular polysaccharides (IPS) in the presence of sugar. EPS and IPS influence the cariogenicity of dental biofilms by at least two pathways: (a) EPS promote bacterial adherence and accumulation on tooth surfaces, and cause biochemical and structural changes in the matrix of the biofilms; and (b) IPS promote lower fasting pH levels during periods of nutrient deprivation, which could result in the selection of cariogenic micro-organisms and caries development [5]. Thus it is understood that without adhering to the tooth structure as a biofilm, the microbes cannot initiate carious lesions on the tooth.

Controlling the frequency of intake of dietary sugar and use of alternative sugars in foods have therefore been recommended as the preventive strategy for public and private in dental caries management [6]. Sugar substitutes are those that cannot be metabolised by the cariogenic organisms, thus cannot lower the biofilm pH on the tooth surface. They are classified as intense sweeteners (noncaloric) like aspartame, saccharin, sulfame, glycyrrhizin and bulk sweeteners (caloric) like sorbitol, xylitol, mannitol [7].

Sorbitol and Xylitol are the most commonly used sugar substitutes. Many studies are available that have shown the anticariogenicity of Xylitol. Xylitol chewing gums are commonly used in the non-operative management of dental caries. Apart from preventing adhesion of the microorganism it also promotes salivation and thus remineralisation, when used in the form of a chewing gum [8]. Extracts obtained from stevia leaves contain glycosides, namely, Stevioside and Rebaudioside-A.These compounds have sweetness intensities of more than 300 times than that of sucrose. Therefore, they are used as sweeteners in foods, drinks and

confectioneries. Studies have found them not to be cariogenic [9]. Honey has been investigated more for use in diabetic patients and obesity. But very few studies have found its effectiveness in caries formation [10].

The production of the acidic by-product during metabolism of the sugar by the microorganism, cause demineralisation of the tooth at the biofilm interface. The depth of demineralisation depends on the ability of the sugars to be metabolised. Honey's cariogenic capacity has been evaluated in a recent study, where it was found to cause lesser demineralisation when compared to glucose [11]. Palm sugar has also been evaluated in a similar way and has shown lesser demineralisation than sucrose [12].

The aim of the study is to evaluate the effect of naturally available sweeteners and unrefined sugars on *S. mutans* biofilm formation using MTT assay and enamel demineralisation using polarised light microscopy**.**

The null hypothesis was there will not be any difference in the cariogenicity of natural sweeteners/ unrefined sugars and sucrose

II. Materials and Methods

This study was conducted at the Department of Centre for interdisciplinary and research facility (CIDRF)in Mahatma Gandhi Medical College and Research Institute , Oral Pathology and Microbiology in Indira Gandhi Institute of Dental Sciences (Pondicherry) and Sri Ramachandra Institute of Dental Sciences (Chennai).

Study design:observational study

Study location: Centre for interdisciplinary and research facility (CIDRF)in Mahatma Gandhi Medical College and Research Institute , Oral Pathology and Microbiology in Indira Gandhi Institute of Dental Sciences (Pondicherry) and Sri Ramachandra Institute of Dental Sciences (Chennai)

Study duration: June 2016 to October 2018

Sample size for demineralization experiment:120 teeth

Sample size calculation: Used G * power analysis Used the mean and SD of the depth of demineralization in micrometre for the honey group and sucrose group from the previous study (11) to calculate the sample size for a particular group. As there are 6 groups, the sample size is estimated as 20 x 6 = 120.

Inclusion criteria:
Caries free human premolars

Exclusion criteria :
1) No development defects seen on the tooth surface
2) No cracks or white spots
3) No Caries

Procedure methodology

1. Biofilm formation assessment:

Bacteria and Culture Conditions*:*

Standard Streptococcus mutans (ATCC 25175) was procured from sigma Aldrich labs (Chennai).

The pure strain of microorganism was cultured in brain-heart infusion broth for 12 h at 37 ° C in a 5% supplemented CO_2 environment.Cells were harvested by centrifugation (800 g, 19 °C, 5 min), washed twice with sterile PBS and re-suspended in the same buffer.The optical density of the cell suspension was adjusted to 0.3 optical density units at 550 nm using a spectrophotometer. The adjusted optical density corresponds to a microbial concentration of 3.65×10^8 cells/ml.

MTT Assay Reagents

MTT stock solution was prepared by dissolving 5 mg/ml 3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide in sterile PBS; PMS stock solution was prepared by dissolving 0.3 mg/ml of N-methylphenazonium methyl sulfate in sterile PBS. The solutions were stored at 2°C in lightproof vials until the day of the experiment, when a fresh measurement solution was prepared by mixing 1 ml of MTT stock solution, 1 ml of PMS stock solution, and 8 ml of sterile PBS. A lysing solution was prepared by dissolving 10% v/v sodium dodecyl sulfate and 50% v/v dimethylformamide in distilled water and stored at 2 °C until the day of the experiment, when it was warmed at 37°C for 2 h before use.

Biofilm Development and MTT Assay

MTT Assay

In a 96 multi well plate, 160µl of sterile BHI broth and 20 µl of bacterial suspension were placed. 20µl of sweetener solutions (10% solution of honey, jaggery,palm sugar, stevia, sucrose and xylitol) were added respectively to 16 wells of 96 well plate. The plate was incubated for 48 h at 37°C in a 5% supplemented CO_2 environment. Then the culture was removed from the wells and gently washed with PBS solution 3 times to remove the non-adherent bacteria.100 µl of MTT assay reagents was then be added to the plates and was incubated at 37°C under lightproof conditions for 3 hours. During incubation, microbial redox systems will convert the yellow salt into insoluble purple formazan. After 3 hrs, the reagent were then be carefully removed and the formazan crystals were dissolved by adding the 100µl of lysing solution to each well. The plates was then be stored for 1 h under lightproof conditions at room temperature. Then 80 µl of the solution was transferred to the wells of a new 96-well plate. Optical density of the solution was measured using a spectrophotometer.

2 . For enamel demineralization assessment

Selection of teeth and preparation of specimen

120 caries-free human premolars were selected and stored in 10% formalin solution to disinfect them and prevent the growth of bacteria, or else it would remain viable within the root canals of the teeth. No developmental defects, cracks, caries, or white spots were found on the buccal enamel surface of the teeth. All the remaining soft tissues were removed using a razor blade; the teeth were cleaned using non-fluoridated pumice and polished with prophylactic rubber cups. All surfaces of the teeth were covered with nail polish except the buccal surface.The root portion of the teeth was resected and the root end was blocked with wax.

BHI solution prepared and sterilised in an autoclave. S mutans bacterial suspension was made as mentioned previously.Each tooth was placed in a test tube with 3 ml of BHI broth and incubated in a 37-degree C incubator and assessed after 24 hours for any contamination by evaluating the cloudiness of those samples. Then the tubes were randomly divided into 6 groups (n=20).

Each group was put into new flasks containing 100 ml of six different solutions

Preparation of sample sweeteners

Group 1 -10 g of xylitol +10 ml distilled water+90 ml sterilized BHI broth
Group 2 -10 g of sucrose +10 ml distilled water+90 ml sterilized BHI broth
Group 3 -10 g of honey + 90 ml sterilized BHI broth
Group 4 -10 g of jaggery+10 ml distilled water+90 ml sterilized BHI broth
Group 5 -10 g of palm sugar+10 ml distilled water+90 ml sterilized BHI broth
Group 6 -10 g of stevia +10 ml distilled water+90 ml sterilized BHI broth

About 1.5×10^8 cells of Streptococcus mutans ATCC 2517 (equivalent to 0.5 McFarland units) was added to each flask. Every day 2ml of the old solution was removed and fresh 2 ml solution was added. The removed solution was checked for no contamination by growing in agar medium. After 21 days, the teeth were taken and The samples were mounted in self-cure acrylic resin and sectioned buccolingually using a hard tissue microtome to obtain sections of approximately 300 mm in thickness. A final polishing was done to get a thickness of 100 mm using high-capacity grinding microtome for histological examination .The sections were immersed in water (with refractive index of 1.33) for evaluation under Polarised light microscopy.The demineralization depth was measured at 3 different depths of each section and the average of the three representative measurements was taken and considered as the lesion depth in micro meter.

Statistical analysis

To compare six varieties with respect to absorbance the appropriate statistical tool is One Way ANOVA. Upon observation of significance of p-value (i.e., p-value <0.05), a multiple comparison test, by name Tukey's test is performed for knowing which pair of groups differ significantly.

III. Results

For biofilm assessment

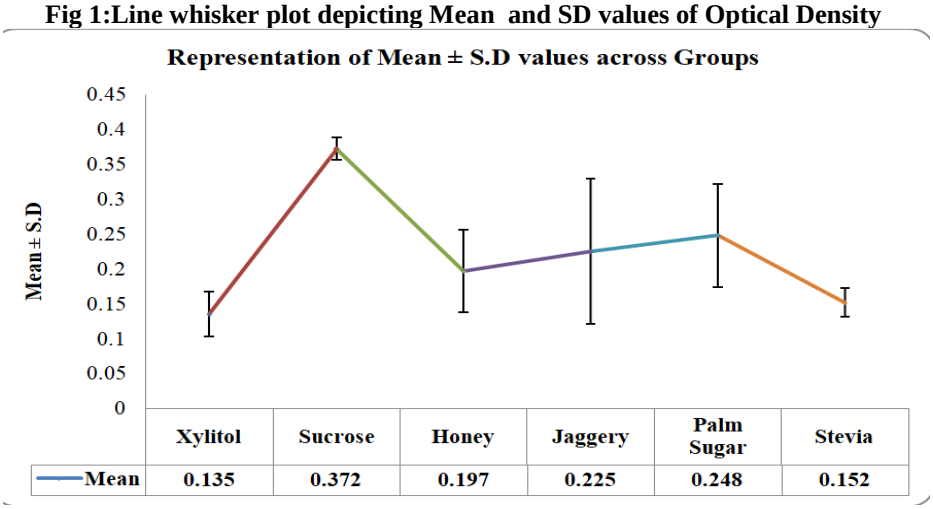
Bacterial colonization was observed in all samples, but to a greater extent in the wells containing the 10% sucrose solution. One-way ANOVA showed significant differences among the 6 means ($p < 0.01$).

With the Tukey's test, it is noticed that mean absorbance level do not differ between Xylitol and Stevia, Sucrose and Palm Sugar, Honey and Jaggery but these pairs of groups differ between them (this is shown by defining superscripts for mean absorbance value (that is same superscript do not differ and different superscripts differ significantly)(**Table 1).**

Table 1:Mean and SD Values of Optical Density for Biofilm Assessment

Group	N	Mean	Std. Deviation	F-test (p-value)
Xylitol	16	0.135[a]	0.032	32.346 (0.000*)
Sucrose	16	0.372[c]	0.016	
Honey	16	0.197[b]	0.059	
Jaggery	16	0.225[b]	0.104	
Palm sugar	16	0.248[c]	0.074	
Stevia	16	0.152[a]	0.021	

Fig 1:Line whisker plot depicting Mean and SD values of Optical Density



2 For Enamel Demineralization

The mean depth of enamel demineralization for all groups were calculated at three points(**Table 2-4**).

Table 2:Depth of demineralization induced by xylitol and sucrose

GROUP 1					GROUP 2			
XYLITOL					SUCROSE			
DEPTH OF DEMINERALIZATION				AVERAGE	DEPTH OF DEMINERALIZATION			
SL NO	1st	2nd	3rd		1st	2nd	3rd	AVERAGE
1	161.471	165	139.846		240.917	234.691	243.666	239.758
2	128.86	150.27	185.321	155.439	207.889	311.517	231.019	250.159
3	120.785	112.929	122.413	154.817	204.793	349.986	203.027	252.602
4	137.641	150.987	166.979	118.709	288.141	272.868	266.308	275.772
5	119.365	119.281	130	151.869	247.168	312.36	333.216	297.581
6	119.474	132.612	130.25	122.882	297.551	276.016	396.011	322.193
7	171.026	140.456	128.16	127.445	343.274	375.396	283.623	334.098
8	163.768	141.287	121.824	146.548	303.638	278.871	223.958	268.822
9	126.463	118.186	112.73	119.126	188.809	269.416	369.11	275.778
10	140.616	105	136.821	127.479	204.793	349.986	203.027	252.602
11	140.057	136.059	128	134.705	239.44	252.815	236.238	242.831
12	140	142.436	130.231	137.556	257.295	262.84	251.665	257.266
13	132.061	148.216	160.2	146.826	240.687	297.658	274.749	271.031
14	128.062	124.258	120.599	124.306	220.907	252.539	244.229	239.225
15	136.821	127.122	134.104	132.682	286.356	308.415	302.417	299.063
16	133.507	132.966	123.223	129.899	290.93	294.944	272.617	286.164
17	124.258	140.057	148	137.438	224.036	308.649	265.481	266.055
18	152.84	160.2	148.216	153.752	297.859	293.394	280.941	290.731
19	165.747	162.432	164	164.059	253.513	258.157	252.606	254.759
20	151.42	153.31	154.506	153.079	324.197	342.041	298.931	321.723
Mean				139.045	Mean			**274.910**
+_SD				13.67	SD			**28.46**

Table 3:Depth of demineralization induced by honey and jaggery

GROUP 3					GROUP 4			
HONEY					JAGGERY			
DEPTH OF DEMINERALIZATION				AVERAGE	DEPTH OF DEMINERALIZATION			
SL NO	1st	2nd	3rd		1st	2nd	3rd	AVERAGE
1	135.93	154.434	216.375	168.913	150.12	129.035	156.259	145.138
2	154.871	157.035	171.945	161.284	153	159.708	140.872	152.193
3	147.122	132.034	141.51	140.222	164.454	165.082	158.532	162.689
4	159.706	150.03	145.121	151.619	179.524	173.118	160.016	170.886
5	137.641	136.854	126.143	133.546	192.842	261.276	132.136	195.418
6	180.1	162.111	200.26	180.824	232.476	203.337	182.508	206.107
7	159.454	177.025	144.031	160.17	215.102	236.315	250.801	234.073
8	169.095	226.753	179.825	191.891	158.405	167.839	135.532	153.925
9	231.078	201.201	168	200.093	135.3	160.016	174.645	156.654
10	223.233	142.271	169.201	178.235	211.367	193.884	205.078	203.445
11	146.697	145.602	141.873	144.724	153.883	115.447	104.995	124.775
12	120	116.069	136.528	124.199	159.248	174.951	169.517	167.906
13	130.231	106.733	124.193	120.386	176.409	156.205	193.039	175.217
14	139.714	134.104	109.252	127.69	152.21	156.205	156.051	154.822
15	136.059	152.21	144.056	144.108	152.21	148	160	153.403
16	140.513	141.697	133.507	138.572	219.126	224.321	177.539	206.099
17	152.053	152.315	157.785	154.051	146.697	136.528	104.307	129.178
18	159.437	165.447	134.948	153.277	160.05	148.054	140	149.368
19	160	148.054	150.625	152.893	152	144	132	142.667
20	140	148.054	160.798	149.617	222.639	177.088	216.333	205.353
Mean				153.815	Mean			169.465
+_SD				21.70	+_SD			29.53

Table 4: Depth of demineralization induced by palm sugar and stevia

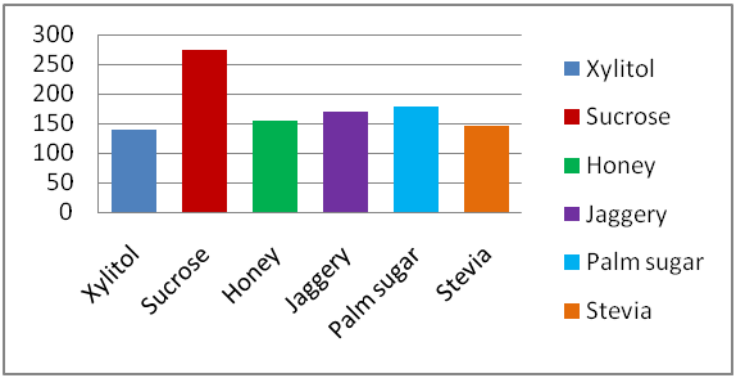
GROUP 5					GROUP 6			
PALMSUGAR					STEVIA			
DEPTH OF DEMINERALIZATION				AVERAGE	DEPTH OF DEMINERALIZATION			
SL NO	1st	2nd	3rd		1st	2nd	3rd	AVERAGE
1	150.03	127.279	152.411	143.24	218.536	224.84	178.216	207.297
2	236.947	149.773	82.989	156.57	175.57	157.664	192.234	175.156
3	225.18	178.62	139.389	181.129	179.8	135.565	167.705	161.023
4	160.801	177.229	171.237	169.755	151.463	148.098	158.319	152.627
5	263.795	200.821	189.452	218.023	153.792	153.235	158.773	155.267
6	217.702	209.163	230.376	219.08	146.509	135.665	103.619	128.598
7	244.182	205.407	213.063	220.884	152.971	145.523	131.214	143.236
8	235.915	226.515	222.122	228.184	159.48	149.94	139.589	149.67
9	211.473	150.748	134.833	165.685	153.938	113.208	108.706	125.284
10	168.241	318.057	174.026	220.108	218.536	224.84	178.216	207.297
11	220.036	144	168.428	177.488	132.034	117.154	120	123.063
12	240.3	200.36	188.68	209.78	123.329	84.853	123.11	110.43
13	172.418	229.713	180.4	194.177	160.016	138.975	147.733	148.908
14	187.489	176.59	191.207	185.095	165.109	96.047	75.06	112.072
15	152.053	152.21	168.19	157.484	150.987	149.037	161.276	153.767
16	132	148	200.04	160.013	151.433	145.245	131.59	142.756
17	130.231	134.164	117.712	127.369	123.037	108.167	121.491	117.565
18	140.057	164.195	142.042	148.765	147.031	129.035	144.031	140.032
19	160.05	176	144.056	160.035	105.043	135.033	123	121.025
20	150.572	134.104	141.873	142.183	150.987	149.037	161.276	153.767
Mean				179.25	Mean			146.44
+_SD				31.09	+_SD			27.16

The mean depth of demineralization for group 1 (xylitol) was 139.045,group 2(sucrose) was 274.910,Group 3(honey) was 153.815,Group 4 (jaggery) was169.465,Group 5 (palmsugar) was 179.252 and group 6(stevia) was 146.442.

One-way ANOVA showed a statistically significant difference between all the groups ($P < 0.001$)

Intergroup comparison with Tukey's test showed no significant difference between group 1(xylitol),group 3(honey) and group 6 (stevia) ($P>0.05$).However group 2 (sucrose) showed significant difference with all other groups& Group 1 (xylitol) with group 4 &5. and group 6(stevia) with group 5(palmsugar) ($P<0.001$)

A graphical representation of the mean depth of demineralization for all the six experimental groups is shown in **(Fig 2)**.



In the present study, xylitol group exhibited the least depth of enamel demineralization, followed by stevia, honey, jaggery ,palm sugar and sucrose groups.There was no statistically difference between xylitol and stevia and honey groups but the former two were significantly better than Palmsugar and sucrose groups.

IV. Discussion

Dental caries is the most prevalent chronic diseases worldwide, and a costly burden to health care services [1].It is the result of interaction among three basic components a tooth substrate, acidogenic bacteria, and diet rich in fermentable carbohydrates for the bacteria to metabolize [13]. The ability of M.streptococci to tolerate changes in the environmental pH and to form biofilms on the tooth surface allows them to survive and persist in the oral ecosystem [14]. Bacterial action on fermentable dietary carbohydrates leads to the production of acids, diffusion into the teeth, demineralization, and ultimately the formation of dental caries [11].

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 [5] and is easily fermentable when compared to other starches [15].To avoid the role of sucrose on the virulence factors of S. mutans, the use of sugar substitutes are 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.Here, S. mutans treated with sweeteners containing, xylitol,stevia, honey, jaggery, palm sugar showed less biomass and demineralization than sucrose.

Herbal interventions in dental caries management has 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 [9]. It has also been widely used for its antibacterial effect and is widely used in pharmaceutical industry.Inspite of its sweeteness that is 300 times more than sugar,it is found to be not a cariogenic substance. As the extract of stevia has antibacterial and anti fungal effect,it also raised 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%) and carbohydrates (63%). Stevia is found to be effective in reducing the cariogenic microbial count and enamel demineralization in the current study.This is in accordance with previous in vitro and in vivo studies [9].

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 [16]. Honey has antibacterial activity against cariogenic

bacteria such as S. mutans and Lactobacillus [17]. 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 [18].

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 [19]. The results of this study indicated that honey has fewer caries activity than sucrose, palmsugar and jaggery. In the present study, the effect of honey on enamel demineralization confirms with (ahmadi&razdan)study [11,12].

Jaggery is a sugarcane based traditional Indian sweetener. It contains upto 50% sucrose,20% invert sugars, vitamins,(0.6%-1.0% minerals; important among them are iron (11mg%), calcium (0.4%), magnesium and phosphorous (0.045%), protein (0.25%), and fat (0.05%). It also have higher medicinal and nutritional values and easily available to the rural people.It is better to consume jaggery as compared to sugar for iron content, minerals, and vitamins present in it along with sucrose. Healthy people can substitute sugar with jaggery[20].The present study results showed that it is less cariogenic when compared to sucrose and palmsugar.

Palmsugar is a sweetener derived from Palmyra palm, a multipurpose tree with great utility is believed to be a native of tropical Africa, although it grows extensively in the different parts of India, Sri Lanka and Myanmar and Bangladesh [21]. The main components of palm sugar are sucrose(70-80%) with glucose (3-9%) and fructose(3-9%) [22]. It is rich source of calcium, phosphorus, proteins,fat.and water. It is known to have medicinal qualities and is widely used in Indian Medical Systems. In previous literature,a single invitro study conducted regarding its cariogenic potential and it states that though it is cariogenic, it has less demineralization potential than sucrose [12].This is in accordance with the current study results which showed that palm sugar cause less biofilm formation and enamel demineralization depth than sucrose but more than jaggery, honey and statistically significant difference showed between stevia and xylitol .

Mechanisms to explain the lower demineralizing potential of the commercial and natural sugars tested here may be due to lack of metabolization by S. mutans. Substitutes for sugars, such as sugar alcohols, are fermented poorly or not at all by oral bacteria, and as a result, they have negligible cariogenic potential [23]. Streptococcus mutans do not have enzymes to utilize Xylitol as a source of energy for acid production or for synthesis of extracellular polysaccharides. Experimental studies in rats have demonstrated an extremely low caries rate in the presence of a Xylitol-containing diet [24]. It is known that (a) xylitol in chewing gum and sweets influences the quantity of plaque and saliva, that (b) xylitol reduces enamel demineralization in vitro and that (c) xylitol forms complexes with calcium ions [14].Here among the other sweeteners, the negative control xylitol showed least biomass and least demineralization .The previous experiments with xylitol confirms the results obtained in the current study.

In this current study, xylitol showed least biomass and demineralization followed by stevia, honey,jaggery,palmsugar and sucrose. Taken together, these results suggest that artificial and natural sweeteners have lower cariogenic and demineralizing potential than sucrose. The former seems to derive from the incapacity of S. mutans biofilms to metabolism the products with the same efficiency the bacterium ferments sucrose, despite the presence of other fermentable carbohydrates.

Results of the present study indicate that the artificial sugars such as xylitol and natural sweeteners like stevia, honey, jaggery and palmsugar are less cariogenic, and the use of artificial sugars should be carefully recommended.

V. Conclusion

Within the limitations of the present study, it may be concluded that all the experimental sweeteners studied here was found to be less cariogenic and have less demineralising potential than sucrose .This is in acceptance with the research hypothesis of this study. Eventhough xylitol exhibited the least mean depth of enamel demineralization, it cannot be statistically proved better than the stevia and honey group.Thus the outcome of this study can be utilized for future dietary purposes.

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