E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.48047/ijprt/16.01.151

Research Article

Comparative Analysis of Salivary pH and Streptococcus mutans Levels following natural sugar substitutes. An in vivo study in and around Raichur District.

Dr. K. M. Parveen Reddy[1], Dr. Anitha K[2], Dr. Bhaskar Kurre[3], Dr. Anant A. Takalkar[4]

[1]Professor, Paediatric and Preventive Dentistry, Navodaya Dental College, Raichur, Karnataka, India.
[2]Professor, Department of Physiology, Navodaya Medical College and Research Center, Raichur, Karnataka, India.
[3]Assistant Professor Cum Statistician, RVM Institute of Medical Sciences and Research Center, Laxmakkapally, Siddipet District.
[4]Professor, Department of Community Medicine, MIMSR Medial College, Latur, Maharashtra, India.
Name of the corresponding author: **Dr. K. M. Parveen Reddy**

Abstract:
Background: Dental caries is a chronic, multi factorial disease most prevalent among children[1] the present concept of etiology of dental caries is mainly due to local role of fermentable carbohydrates like sucrose fructose etc. Which alter the Ph of saliva and favors the growth of bacteria responsible for enamel demineralization. **Objective:** This study will be conducted to compare the effect of natural alternatives for sugar like stevia, jiggery, honey, dates syrup on the pH and S. mutans count in the saliva. **Materials and methods**: The study will be done in children of age group 7-12 years who have normal teeth with no active caries. 100 subjects will be included in the study; they will be randomly divided into 5 groups each with 20 subjects. One group will be given distilled water to rinse the mouth. Other 4 groups will be given the solutions of stevia, jiggery, honey and dates syrup one solution to each group. Sample of saliva will be collected from each group after rinsing. Ph of the saliva and the S mutans count in saliva will be evaluated in each group.

INTRODUCTION:

Dental caries is most prevalent in childhood and adulthood, which is a chronic and multifactorial disease.[1] prevalence of dental caries in children between 2-11 years had shown increase from 42% in 1999-2004 to 49% in 2018[5] At present the prevalence is 54.16%. [2-4]

Multiple risk factors are associated with dental caries like genetic, environmental, socio-economic conditions, oral health and dietary habits.[5,6] dental caries is mainly caused due to interaction of oral microbes with dietary constituents.[7] Many studies have established that sugar plays a important role in causing caries as they are the sole substrate for synthesizing extracellular glycans in dental plaque.[8] as per the current concept of etiology of dental caries the fermentable carbohydrates like sucrose (due to acidic p^H) cause demineralization of the enamel. Some of the factors to be considered in the cariogenicity of carbohydrates is frequency of its consumption which has a greater significance then its quantity.[7] Another important factor is prolonged retention of the food specially the processed foods like chocolates and biscuits which has more cariogenic effect [9] as it increases the duration of exposure to low p^H.

Fermentation of sucrose results in low p^H which shifts the balance of resistant plaque microflora to more acidogenic and acid tolerant gram +ve bacteria (streptococcus mutans)[10]. the extracellular polysaccharide (EPS) synthesized from sucrose by the action of bacteria, promotes the changes in composition of biofilm matrix *i.e* the biofilm formed in the presence of sucrose has low concentration of Calcium and phosphate which are important ions involved in demineralization, remineralization of enamel and dentin.[11]

From various studies, knowledge of concept of dental caries. It is clear that dental caries is a continuous process resulting from repeated cycles of demineralization and remineralization.[12]occurence of caries and its progression can be prevented without need for surgical intervention by modifying one of the important etiological factors, sugar intake in diet. It can be achieved by controlling the frequency of dietary sugar and by replacing it by alternative sugars.[13]

Sugar substitutes are sweet and have low calories. They are not metabolized by caries causing microbes and so p^H of dental plaque is not reduced. Replacing the sugars with natural sweeteners like stevia, jiggery, honey and dates syrup may to some extents have a potential role in reducing the risk of developing caries and maintain better oral health[14]

Stevia is a zero-calorie sweetener extracted from the leaves of plant steviarebaudiana. Stevia is a heat stable, p^H stable and non-fermentable.

Jaggery is a natural sweetener obtained from sugarcane like sugar. But it is not refined. One of the studies done by Takara K et.al has shown that the phenolic compounds which were extracted from the sugarcane malasses have shown antibacterial activity against Streptococcus mutans and streptococcus sobrinus, thus have the potential to reduce caries.[15]

Honey is a natural sweet nector collected by bees from various plants. Presence of various factors like high osmotic pressure low p^H, phenolic acids, lysozyme, flavonoids and natural antioxidants has made honey to have anti-inflammatory and antibacterial activity.[16]

Materials and methods

The study was conducted in various dental clinics in and around Raichur. written informed

Consent was obtained from the parents or the legal guardians of all the participating children who were selected for the study based on inclusive and exclusive criteria.

Inclusive criteria

- Children of age group 7-12 years
- Children with no active caries
- Healthy children without any systemic diseases
- No h/o any preventive dental treatment

Exclusive criteria

- Children having dental caries and undergoing treatment
- Having any painful oral conditions
- Using any mouthwash for oral hygiene
- Children with any systemic disease

A total of 100 children were selected and randomly they were assigned to one of the groups

Group A to Group E, Group E is a control group, each group receiving a different mouth rinse solution.

Group A – stevia dissolved in distilled water

Group B – jaggery in distilled water

Group C – honey in distilled water

Group D – dates syrup in distilled water

Group e – only distilled water control group

Preparation of mouth rinse solution:

The solution for mouth rinse for experiment groups was prepared fresh before the use. Commercially available stevia, jiggery, honey and dates syrup was used. 10ml of each one was mixed with 90ml of distilled water, to make the respective mouth rinse solution.

Collection of samples:

The study subjects were asked to abstain from eating at least one hour before coming to sample collection. Sterile containers were used for collecting saliva

Solutions for rinsing were prepared freshly and given to the subject. The subjects were instructed to rinse the mouth with given solution for about one minute. Then they were asked to spit it out into the sterile containers given.

The p^H of the collected saliva sample was evaluated using the p^H strip. Same sample was also used to evaluate the S. Mutans count using the commercially available kit (Gc saliva check mutans kit)

Results:

Table 1: Description of POST RINSE pH Levels Across Study Groups.

POST RINSE pH	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Mini	Maxi	F Value	P Value
				Lower Bound	Upper Bound	mum	mum		
Group - I (Stavia)	20	7.31	0.101	7.265	7.359	7.13	7.46	1158.2	**<0.001**
Group - II (Jaggery)	20	6.12	0.073	6.084	6.152	6.01	6.23		
Group - III (Honey)	20	5.47	0.063	5.435	5.495	5.35	5.59		
Group - IV (Distilled water)	20	7.28	0.093	7.24	7.327	7.13	7.44		
Group - V (Dates Syrup)	20	5.12	0.246	5.008	5.239	4.49	5.49		

The post-rinse salivary pH levels of the study participants across the five intervention groups are summarized in the above table. Each group comprised 20 samples. Group I (Stevia) demonstrated a mean post-rinse pH of 7.31 ± 0.10, with values ranging from 7.13 to 7.46. The 95% confidence interval (CI) for the mean pH in this group ranged from 7.265 to 7.359, indicating consistently alkaline pH levels following rinsing. Group II (Jaggery) showed a comparatively lower mean post-rinse pH of 6.12 ± 0.07, with a minimum value of 6.01 and a maximum of 6.23. The 95% CI for the mean pH ranged between 6.084 and 6.152. Group III (Honey) recorded the lowest mean post-rinse pH among all groups (5.47 ± 0.06), with pH values ranging from 5.35 to 5.59 and a narrow confidence interval (5.435–5.495), reflecting minimal variability. In contrast, Group IV (Distilled water) exhibited a higher mean post-rinse pH of 7.28 ± 0.09, with observed values between 7.13 and 7.44. The 95% confidence interval for this group ranged from 7.240 to 7.327, closely resembling the pH levels observed in the Stevia group. Group V (Dates syrup) showed a mean post-rinse pH of 5.12 ± 0.25, with a wider range of values (4.49–5.49), indicating greater variability in pH response following rinsing. One-way analysis of variance (ANOVA) revealed a **highly statistically significant difference** in post-rinse pH levels among the five groups (F = 1158.2, $p < 0.001$). This finding indicates that the type of rinse solution had a significant influence on post-rinse salivary pH

.

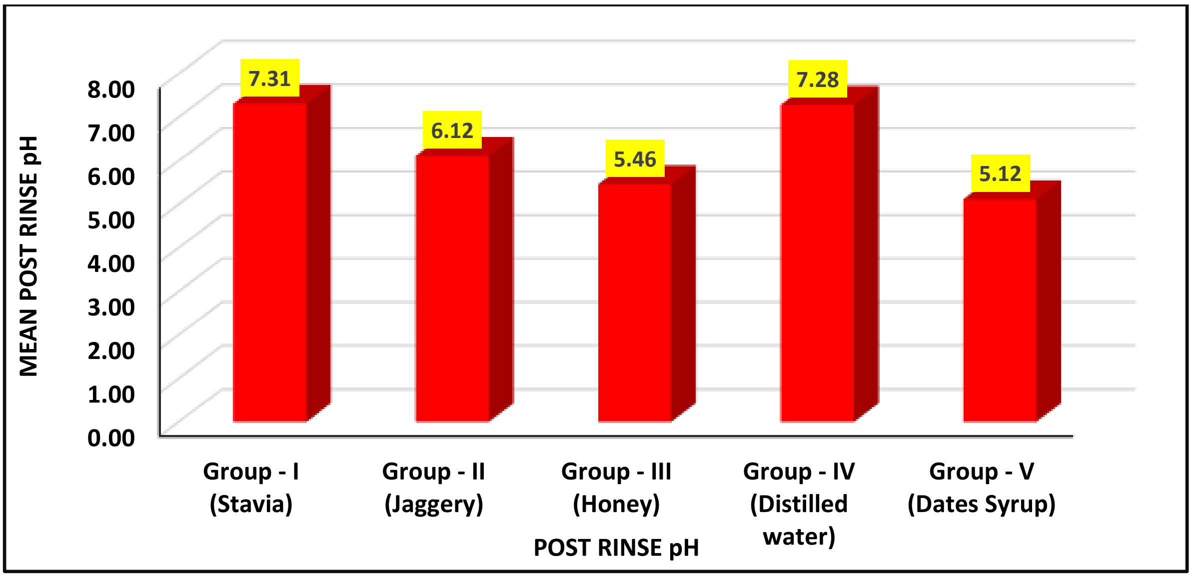

Figure1 : Bar Diagram Showing Mean Post-Rinse Salivary pH Levels Across Study Groups.

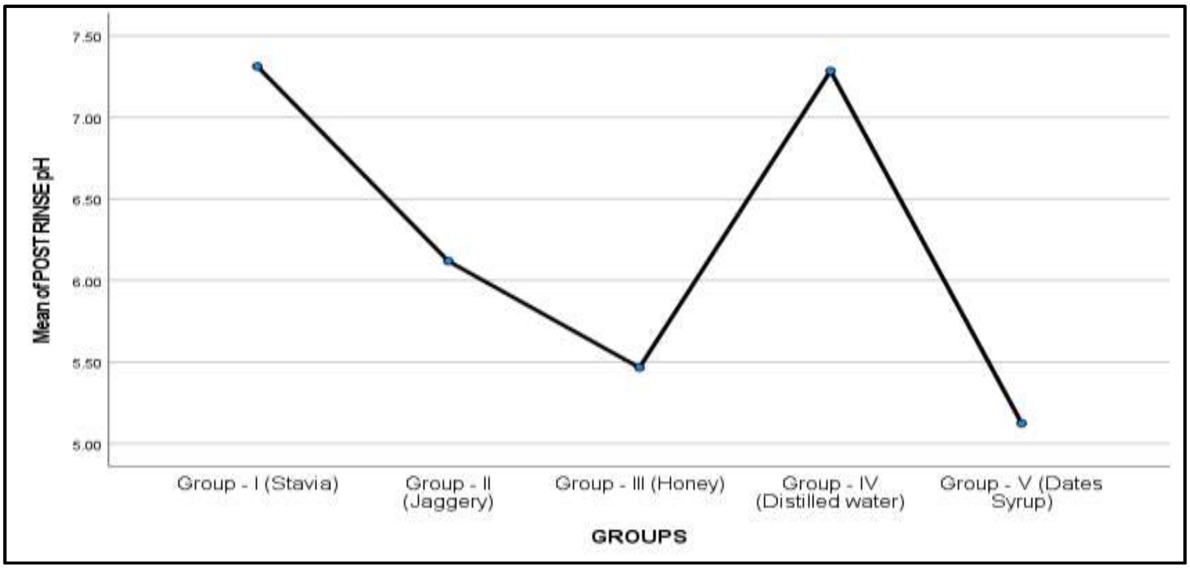

Figure1 :Line Diagram Showing Mean Post-Rinse Salivary pH Levels Across Study Groups.

Table 2: Post-hoc Multiple Comparisons of Mean Post-Rinse Salivary pH Levels Between Study Groups Using Tukey's HSD Test.

Multiple Comparisons						
Dependent Variable:						
Tukey HSD						
(I) GROUPS		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group - I (Stavia)	Group - II (Jaggery)	1.19400*	0.04206	**0**	1.077	1.311
	Group - III (Honey)	1.84700*	0.04206	**0**	1.73	1.964
	Group - IV (Distilled water)	0.0285	0.04206	0.961	-0.0885	0.1455
	Group - V (Dates Syrup)	2.18850*	0.04206	**0**	2.0715	2.3055
Group - II (Jaggery)	Group - I (Stavia)	-1.19400*	0.04206	**0**	-1.311	-1.077
	Group - III (Honey)	.65300*	0.04206	**0**	0.536	0.77
	Group - IV (Distilled water)	-1.16550*	0.04206	**0**	-1.2825	-1.0485
	Group - V (Dates Syrup)	.99450*	0.04206	**0**	0.8775	1.1115
Group - III (Honey)	Group - I (Stavia)	-1.84700*	0.04206	**0**	-1.964	-1.73
	Group - II (Jaggery)	-.65300*	0.04206	**0**	-0.77	-0.536
	Group - IV (Distilled water)	-1.81850*	0.04206	**0**	-1.9355	-1.7015
	Group - V (Dates Syrup)	.34150*	0.04206	**0**	0.2245	0.4585
Group – IV	Group - I (Stavia)	-0.0285	0.04206	0.961	-0.1455	0.0885
(Distilled water)	Group - II (Jaggery)	1.16550*	0.04206	**0**	1.0485	1.2825
	Group - III (Honey)	1.81850*	0.04206	**0**	1.7015	1.9355

	Group - V (Dates Syrup)	2.16000*	0.04206	**0**	2.043	2.277
Group - V (Dates Syrup)	Group - I (Stavia)	-2.18850*	0.04206	**0**	-2.3055	-2.0715
	Group - II (Jaggery)	-.99450*	0.04206	**0**	-1.1115	-0.8775
	Group - III (Honey)	-.34150*	0.04206	**0**	-0.4585	-0.2245
	Group - IV (Distilled water)	-2.16000*	0.04206	**0**	-2.277	-2.043
*. The mean difference is significant at the 0.05 level.						

Post-hoc multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to identify pairwise differences in mean post-rinse salivary pH levels among the five study groups (Table __). The analysis demonstrated statistically significant differences between most group pairs at the 0.05 level. Group I (Stevia) showed significantly higher mean post-rinse pH levels compared to Group II (Jaggery) (mean difference = 1.194, $p < 0.001$), Group III (Honey) (mean difference = 1.847, $p < 0.001$), and Group V (Dates syrup) (mean difference = 2.188, $p < 0.001$). However, no statistically significant difference was observed between Group I (Stevia) and Group IV (Distilled water) (mean difference = 0.0285, $p = 0.961$), indicating comparable post-rinse pH levels between these two groups. Group II (Jaggery) exhibited significantly higher mean post-rinse pH values than Group III (Honey) (mean difference = 0.653, $p < 0.001$) and Group V (Dates syrup) (mean difference = 0.9945, $p < 0.001$), while significantly lower pH levels were observed when compared to Group I (Stevia) and Group IV (Distilled water) ($p < 0.001$). Group III (Honey) demonstrated significantly lower post-rinse pH levels compared to all other groups, with statistically significant differences observed against Group I (Stevia), Group II (Jaggery), Group IV (Distilled water), and Group V (Dates syrup) ($p < 0.001$ for all comparisons). Group IV (Distilled water) showed significantly higher mean post-rinse pH values when compared to Group II (Jaggery), Group III (Honey), and Group V (Dates syrup) ($p < 0.001$), while no significant difference was found in comparison with Group I (Stevia). Group V (Dates syrup) exhibited significantly lower mean post-rinse pH levels compared to Groups I, II, and IV, while demonstrating significantly higher pH levels than Group III (Honey) ($p < 0.001$). Overall, the Tukey HSD post-hoc analysis confirms that the observed overall difference in post-rinse salivary pH among the study groups is primarily driven by significant pairwise differences, particularly between non-cariogenic rinses (Stevia and distilled water) and fermentable carbohydrate-based rinses (Jaggery, Honey, and Dates syrup).

Table 3: Distribution of Decrease/No Change and Increase in *Streptococcus mutans* Count Among Different Study Groups.

Groups	The number of subjects who showed		Chi square Value	P Value
	Decrease/No Change in S. mutans count	Increase in S. mutans count		
Group - I (Stavia)	10	10	39.3	**<0.001**
Group - II (Jaggery)	1	19		
Group - III (Honey)	0	20		
Group - IV (Distilled water)	12	8		
Group - V (Dates Syrup)	0	20		
Total	23	77		

The distribution of subjects showing decrease/no change and increase in *Streptococcus mutans* count across the five study groups is presented in Table __. A total of 100 observations were analysed, of which 23 subjects demonstrated a decrease or no change in *S. mutans* count, while 77 subjects showed an increase. In Group I (Stevia), an equal distribution was observed, with 10 subjects (50%) showing a decrease or no change and 10 subjects (50%) showing an increase in *S. mutans* count. Group IV (Distilled water) demonstrated a higher proportion of subjects with a decrease or no change (12 subjects; 60%) compared to those with an increase (8 subjects; 40%). In contrast, Group II (Jaggery) showed a predominance of increased *S. mutans* count, with 19 subjects (95%) exhibiting an increase and only 1 subject (5%) showing a decrease or no change. Similarly, Group III (Honey) and Group V (Dates syrup) showed an increase in *S. mutans* count in all subjects (100%), with no participants demonstrating a decrease or no change. Chi-square analysis revealed a **highly statistically significant association** between the type of rinse solution used and changes in *S. mutans* count ($\chi^2 = 39.300$, $p < 0.001$). This indicates that the observed differences in *S. mutans* count among the study groups were not due to chance and were significantly influenced by the type of rinse

.

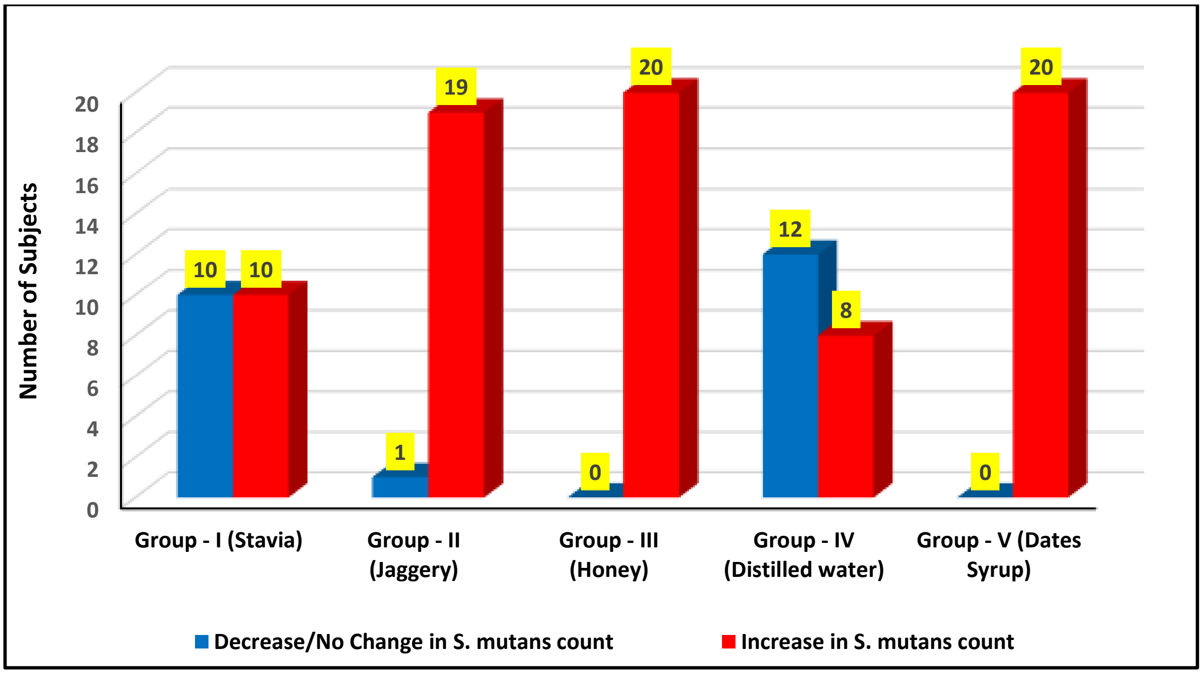

Figure 2:**Bar Diagram Showing Distribution of Decrease/No Change and Increase in** *Streptococcus mutans* **Count Across Study Groups.**

Discussion:

Dental caries remains a major public health concern as it is the most prevalent chronic disease. It is a oral microbial and glucose mediated biofilm dependent oral disease[17] Sucrose plays an important role in the metabolic process of S. mutans hence it is most cariogenic sugar[18] oral bacteria like S. mutans act on fermentable carbohydrates like sucrose to produce acids which diffuse into teeth and cause demineralization leading to caries[19]

To avoid the role of refined sugar in cariogenicity it can be replaced by non-cariogenic or less cariogenic sugar substitutes. This study was conducted to see the effect of natural sugar substitutes like stevia, jaggery, honey and date syrup (distilled water). Guojian Zhang et.al have shown that the stevia extract, steviside effectively inhibited biofilm formation and growth of bacteria and acid production and destroy the structural integrity of biofilm thus decreases the cariogenic property of S. mutans hence can be used as non carigenic sucrose substitute.[20] Winnie Sharma et,al [12] have shown that stevia, jiggery, honey have the ability to reduce caries risk in children. Payal Paul et,al in their study have shown that natural sugar substitutes xylitol, maple syrup and dates syrup can reduce the risk of caries in children.[14]

Our study findings demonstrated that, with stevia there was rise in the salivary pH and decrease in S. mutans count when compared with control group.

Salivary pH was not much increased in groups of post rinse with jaggery, honey and date syrup in comparison with control group.

Conclusion

The present study demonstrates that the type of rinse solution has a significant influence on both post-rinse salivary pH and *Streptococcus mutans* count. Non-cariogenic rinses, particularly Stevia and distilled water, maintained higher post-rinse salivary pH levels, reflecting a more favourable oral environment, whereas fermentable carbohydrate-based rinses such as jaggery, honey, and dates syrup resulted in significantly lower pH values. The observed differences in salivary pH among the study groups were highly statistically significant, with post-hoc analysis confirming marked pairwise variations. In parallel, *S. mutans* count showed a significant association with the type of rinse used, with Stevia and distilled water demonstrating a higher proportion of subjects with decreased or unchanged bacterial counts, while jaggery, honey, and dates syrup were associated with a marked increase in *S. mutans*. Overall, these findings suggest that Stevia and distilled water may be safer alternatives for maintaining oral pH balance and reducing cariogenic bacterial activity compared to fermentable carbohydrate-based rinses.

Conflict of interest: None

Source of funding: Nil

REFERENCES

1. Newbrun E. Dietary carbohydrates: their role in cariogenicity. Med Clin North Am 1979;63(5):1069–1086. DOI: 10.1016/s0025- 7125(16)31660-1
2. Ashley J. Malin, Zhilin Wang, Durdana Khan and Sarah L Mckune. The potential systemic role of diet in dental caries development and arrest: A narrative review. Nutrients 2024, 16, 1463.
3. Fleming E, Afful J, Prevalence of total and untreated dental caries among youth: United States, 2015-2016; NHCS Data Brief. No. 307; National center for health statistics: Hyattsville, MD, USA, 2018.
4. Pitts, N.B.; Zero, D.T.; Marsh, P.D.; Ekstrand, K.; Weintraub, J.A.; Ramos-Gomez, F.; Tagami, J.; Twetman, S.; Tsakos, G.; Ismail, A. Dental Caries. Nat. Rev. Dis. Primers 2017, 3, 17030
5. Janakiram C, Antony B, Joseph J, et al. Prevalence of dental caries in India among the WHO index age groups: a meta-analysis. J Clin Diag Res 2018;12(8): ZE08–ZE13. DOI: 10.7860/JCDR/2018/32669.11956
6. Devan, Induja, Ramnarayanan, Venkitachalam, Janakiram C. Indian journal of public health 66 (suppl); p S3-S11, November 2022/ DOI: 10.4103 / IJPH 107822
7. Bowen WH, Eastoe JE, Cock DJ. The effect of sucrose solutions on the Ph of plaque in caries active monkeys. Arch oral Biol, 1966 Aug, p- 833-837
8. The Tooth Decay Process: How to Reverse It and Avoid a Cavity. Available online: https://www.nidcr.nih.gov/health-info/tooth-decay/more-info/tooth-decay-process (accessed on 11 January 2024)
9. Hancock, S.; Zinn, C.; Schofield, G. The consumption of processed sugar- and starch- containing foods, and dental caries: A systematic review. Eur. J. Oral Sci. 2020, 128, 467– 475
10. Marsh PD, Are dental diseases examples of ecological catastrophies? Microbiology 2003; 149(pt 2): 279-294 doi:10.1099/mic
11. Paes Leme, A.F.; Koo, H.; Bellato, C.M.; Bedi, G.; Cury, J.A. The role of sucrose in cariogenic dental biofilm formation–new insight. J. Dent. Res. 2006, 85, 878–887
12. Jayadevan A, Chakravarthy. comparative evaluation of cariogenic potential of natural and unrefined sweetenerson streptococcus mutans biofilm formation and enamel demineralization – In vitro study. Journal of Dental and Medical sciences, vol 18, issue 3 Ser, 8 (march 2019) p 63-70.
13. Durso S C, Vieiera, L M, Cruz J N S, Sucrose substitutes affect the cariogenic potential of Streptococcus mutans biofilms: Caries Research 48(3): 214-222
14. Payal paul, Sandeep Kumar, Shambhavi Dixit, A Comparative in vivo study of natural sweetening agents and their effects on salivary pH levels among children

visiting dental hospitals in North India. Biomedical informator vol 21(10)

15. Akara K, Ushijima K, Wada K, et al. Phenolic compounds from sugarcane molasses possessing antibacterial activity against cariogenic bacteria.J Oleo S2007;56(11):611–614. DOI: 10.5650/jos.56.61
16. Mammary MA, Meeri AA. Habari MA, Antioxidant activitiesa and total phenolies of different types of honey. Nutr Res 2002; 22; 1041-7
17. Pitts N B, Zero DT/ Marsh P D, et.al Dental caries Nat 2017 Rev. Dis, Primers 3; 17030, doi; 10,1038
18. Shelham A, and James W P T. Diet and dental caries. J Dent Res.2015; 94, 1341-1347, doi: 10.1177/0022034515590377.
19. Ahmadi-Motamayel F. Rezaei-Soufi, Kiani L, Alikhani M Y, Pooralajal J, Effect of honey, glucose and fructose on the enamel demineralization depth.J ournal of Dental sciences 2025; 8(2) 47-50.
20. Rong Ma, Zhang Y, Wang Y and Chu J. Inhibition of streptococcus mutans biofilm formation and virulence by natural extract of stevioside, Front microbial, 2025; 16;1675322 doi; 10,3389/fmic 2025, 1675322.