How to Cite:
Jain, A., Jain, D., Jain, S., Jaiswal, S., & Goyal, R. K. (2022). Effect of chewing Azadirachta indica (neem) and Ocimum sanctum (tulsi) leaves on salivary acidogenicity: A comparative study. *International Journal of Health Sciences*, *6*(S2), 12436–12442. https://doi.org/10.53730/ijhs.v6nS2.8301

Effect of chewing Azadirachta indica (neem) and Ocimum sanctum (tulsi) leaves on salivary acidogenicity: A comparative study

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***Abstract*---**Background: Ocimum sanctum (tulsi) and Azadirachta indica (neem) leaves has been suggested as an antibacterial mouthwash for youngsters. Despite the fact that these herbal plants are having anti-cariogenic property, there are no research on their ability to reverse the cariogenic impacts of other diets.Aim: The purpose of this study was to see if chewing of O. sanctum and A. indica leaves following a cariogenic stimulus caused salivary pH reversal.Methods and Materials: Participants were randomly assigned to one of two groups: Category A using A. indica plant leaves or Category B using O. sanctum leaves. Salivary samples were obtained at 5 minute, 30 minute, and 60 minute intervals after chewing out the herb leaves and completely expectorating to assess the salivary pH. A

digital pH metre (HI 2211 pH/ORP Meter, Rhode Island, USA) was used to measure salivary pH, which was adjusted with standard sample solution of pH 4.0 and 7.0.Results: The salivary pH of both category A and category B children fell significantly after eating chocolate at 5 and 30 minutes and gradually restored to initial level after about 60 minutes. When the categories were compared, A. indica leaves showed the greatest increase in salivary pH ($P < 0.05$), although the difference was only statistically significant after 30 minutes.Conclusion: Chewing A. indica leaves and O. sanctum leaves has been proven to enhance pH reversal after a cariogenic exposure in our research. This finding, together with the anticariogenic effects of these two herbal plants on oral health, leads us to assume that children can be recommended to use these leaves in between meals following caries exposure.

***Keywords*---**dental caries, effect chewing, Azadirachta indica, Ocimum sanctum.

Introduction

The most frequent chronic oral condition impacting children today is dental caries. Dietary fermentable carbohydrates that stay in the mouth for a long time lower plaque pH, resulting in dental caries. As a result, it is recommended that cariogenic foods be cleared out from mouth as quickly as possible by consuming preventive foods. Milk products, cheese, unprocessed green vegetables, and tea have all been demonstrated to be caries-protective foods.[1,2] The research community is continually looking for a caries-preventive material that is locally available, affordable, effective, and culturally acceptable. Chewing the leaves of Azadirachta indica (Neem) plant and Ocimum sanctum (Tulsi) plants has been shown to be helpful home treatments for infectious diseases, dental problems, bleeding, and painful gums.[3,4]

Streptococcus mutans, the microbe that causes dental caries, was found to have antibacterial action in the leaves of A. indica and O. sanctum. Streptococcus mutans bacteria, Streptococcus sanguis bacteria, Streptococcus mitis bacteria, and Lactobacillus acidophilus bacteria have all been shown to be susceptible to O. sanctum leaf extract. After 5 minutes and 30 minutes of chewing leaves, the pH of the plaque increased significantly.[5,6] After more investigation, some authors believe O. sanctum extract can be suggested as an antibacterial mouthwash for youngsters. Despite the fact that these herbal plants are having anti-cariogenic property, there are no research on their ability to reverse the cariogenic impacts of other diets.[7] The purpose of this study was to see if chewing these leaves following a cariogenic stimulus caused salivary pH reversal.

Materials and Methods

After receiving ethical approval for the trial as from the ethical committee of institution, sixty children without caries between the ages 8–10 years were chosen

at random. Children with dental cavities, systemic disorders, uncooperative behaviour, and those who refused to take part in this study were not allowed to be involved. Participants were randomly assigned to one of two groups: Category A using A. indica plant leaves or Category B using O. sanctum leaves. All subjects received oral prophylaxis 24 hours before the study. They were told not to eat or drink for at least two hours before the test. The study participants were encouraged to sit comfortably in the dental chair and relax. With the head tilted forward, approximately one mL of unstimulated form of saliva was placed in a sealed plastic container, and the initial pH of saliva sample was recorded. After eating a chocolate bar (Cadbury Diary Milk chocolate Bar having weight of 13 g), the children's saliva was collected at 5-minute, 30-minute, and 60-minute intervals to estimate salivary pH after an acidogenic challenge. The children were then requested to carry out chewing of five to six new A. indica leaves for one minute with 5 mL of water, while category B study participants chewed 5 to 6 fresh O. sanctum plant leaves for one minute with five mL of water.

Salivary samples were obtained at 5 minute, 30 minute, and 60 minute intervals after chewing out the herb leaves and completely expectorating to assess the salivary pH. All of the salivary samples were sent to experimental lab right away, and indeed the pH of salivary samples was determined as soon as possible, but no later than 30 minutes afterwards. A digital pH metre (HI 2211 pH/ORP Meter, Rhode Island, USA) was used to measure salivary pH, which was adjusted with standard sample solution of pH 4.0 and 7.0. After each analysis, the electrodes were cleaned with the help of distilled water then dried with absorbent paper. To compare the data within the groups, a statistical analysis was performed using the Student's t test. The significance level was set at $P<0.05$ statistically.

Results

The salivary pH of both category A and category B children fell significantly after eating chocolate at 5 and 30 minutes and gradually restored to initial level after about 60 minutes [Tables 1 and 2]. When leaves of A. indica were chewed after the chocolate challenge in category A children, the pH raised to statistically meaningful values after 5, 30, and 60 minutes, with a value higher than the base value at 60 minutes [Table 3]. After chewing O. sanctum leaves for 5 and 30 minutes after the chocolate challenge, pH climbed to statistically significant levels in category B children. [Table 4] The value at 60 minutes was somewhat lower than the baseline value, but not statistically significant. When the categories were compared, A. indica leaves showed the greatest increase in salivary pH ($P <0.05$), although the difference was only statistically significant after 30 minutes [Table 5].

Table 1: Assessment of pH values after consuming chocolate in Category A children (Azadirachta indica [Neem])

	Minimum–maximum	Mean ± SD	Difference	t	P
Baseline pH values	6.58–7.94	7.37±0.48	-	-	-

After consuming chocolate					
5 min	5.41–7.47	6.76 ± 0.62	0.662	11.822	<0.001*
30 min	6.32–7.47	6.81 ±0.47	0.408	7.437	<0.001*
60 min	6.56–7.91	7.27 ±0.51	0.041	1.412	0.474

Statistically significant. SD: Standard deviation

Table 2: Assessment of pH values after consuming chocolate in Category B children (Ocimum sanctum [Tuls)])

	Minimum–maximum	Mean ± SD	Difference	t	P
Baseline pH values	6.24–8.67	7.56±0.67	-	-	-
After consuming chocolate					
5	5.27–7.97	6.82±0.77	0.698	8.536	<0.002*
30	6.22–7.82	6.70±0.52	0.413	3.657	0.004*
60	6.27–7.24	7.33±0.61	0.089	2.078	0.066

Table 3: Assessment of pH values in Category A children after chewing Azadirachta indica leaves following a chocolate challenge

	Minimum–maximum	Mean ± SD	Difference	t	P
Baseline pH values	6.67–7.98	7.14±0.48	-	-	-
After consuming chocolate					
5 min	5.41–7.47	6.79±0.63	-	-	-
30 min	6.45–7.49	6.81 ±0.48	-	-	-
60 min	6.71–7.10	7.29±0.51	-	-	-
After chewing Azadirachta indica leaves following chocolate challenge (min)					
5 min	6.94–8.22	7.57±0.48	0.92	7.579	<0.001*
30 min	6.56–8.05	7.49±0.40	0.59	5.347	<0.001*
60 min	6.50–7.64	7.36±0.28	0.19	2.794	0.012*

Table 4: Assessment of pH values in category B children after chewing Ocimum sanctum leaves following a chocolate challenge

	Minimum–maximum	Mean ± SD	Difference	t	P
Baseline pH	6.43–8.68	7.45 ±0.68	-	-	-
After consuming chocolate (min) Comparison with values after consuming chocolates					
5	5.28–7.97	6.82 ±0.77			

30	6.22–7.82	6.76±0.52			
60	6.98–8.05	7.33±0.72			
After chewing Ocimum sanctum (Tulsi) following chocolate challenge (min)					
5	6.77–8.07	7.49±0.46	0.78	6.124	<0.001*
30	7.01–7.21	7.35±0.31	0.36	2.944	0.022*
60	6.45–7.87	7.36±0.57	0.0367	0.881	0.458

Table 5: Comparison of pH at different time intervals between Group A and B children

			Total	P	
Baseline pH values	7.31 ± 0.48	7.47 ± 0.60	7.35 ± 0.6	0.641	
After consuming chocolate (min)					
5	6.75 ± 0.62	6.82±0.77	6.78±0.69	0.841	
30	6.87 ± 0.46	7.01±0.52	7.02 ±0.49	0.551	
60	7.47±0.51	7.33±0.61	7.24 ±0.55	0.729	
After chewing Azadirachta indica/ Ocimum sanctum following chocolate challenge (min)					
5	7.57±0.48	7.49 ± 0.46	7.53 ±0.46	0.622	
30	7.50±0.51	7.35±0.31	7.48±0.57	0.042*	
60	7.47±0.39	7.36±0.57	7.41±0.49	0.494	

Discussion

The majority of research evaluating the acidogenic propensity of materials were based on measuring plaque pH. However, because salivary pH is known to be a primary component influencing plaque pH, we concentrated on the turnaround of salivary pH just after acidogenic exposure by commonly accessible A. indica leaves and O. sanctum leaves.[8] Furthermore, salivary pH is simple to assess and teach youngsters about the nutritional benefits of foodstuff during diet counselling. Before providing the test foods, the salivary pH was measured as a baseline against which the variation in pH could be evaluated. The pH of saliva should be between 6.2 and 7.6.

The pH in this study varied from from 6.1 to 8.5, confirming previous results that showed a subject-to-subject variance in salivary pH related to caries susceptibility changes. There was a quick drop in salivary pH after the children took chocolate in this study, which is comparable to Stephan's results that sugar-containing diets promote a sharp decrease in oral pH.[9] Somaraj et al., Tayab with associates, Tahmassebi and Duggal conducted other experiments that found a comparable drop in pH. Enamel has a "critical pH" of about 5.5, below which it begins to deteriorate. After the acidogenic challenge, the salivary pH reported in the study was over the threshold pH.[10]

This conclusion could be explained by the children's higher baseline pH, adequate saliva buffering capacity, and improved dental hygiene with less plaque. Furthermore, because chocolate is a high-sugar meal, it has a greater oral clearance than high-starch products, which are more commonly taken by youngsters, resulting in a smaller pH drop. After an acidogenic challenge, salivary pH values were measured at 5, 30, and 60 minutes to record the time it took for salivary pH to rebound to baseline. The pH reverted to baseline after 60 minutes, which is consistent with other research.[11]

To standardise the experimental settings, these children were advised to chew A. indica leaves and O. sanctum leaves for a minute. Because a minimum decline in pH following the chocolate exercise was noticed at 5 minutes, salivary pH measurements were obtained after 5 minutes. After a cariogenic challenge, both A. indica category A and O. sanctum category displayed salivary pH reversal in the current study. After eating A. indica leaves for 5, 30, and 60 minutes, there was a substantial increase in pH. Similar to prior studies, the O. sanctum group demonstrated a statistically significant elevation in salivary pH after 5 minutes and 30 minutes.[12]

The actual method of action, however, is unknown. It has been demonstrated that foods that require a lot of biting produce more voluminous salivary flow with a higher buffering capacity, allowing plaque acids to be neutralised more effectively and facilitating oral evacuation of food debris. This explanation could possibly be linked to the pH variations observed in this study. Diet counselling for the control of the disease includes advice on reducing carbohydrate consumption frequency. Carbohydrate snacks are regularly consumed by children. Although tooth brushing is suggested after sugar exposure, it may not be practicable. As a result, chewing on cariogenic-protective meals following a cariogenic exposure is a viable option.[13]

Conclusion

Chewing A. indica leaves and O. sanctum leaves has been proven to enhance pH reversal after a cariogenic exposure in our research. This finding, together with the anticariogenic effects of these two herbal plants on oral health, leads us to assume that children can be recommended to use these leaves in between meals following caries exposure. To back up our findings, more research with a bigger sample size is required.

References

1. Somaraj V, Shenoy RP, Panchmal GS, Jodalli PS, Sonde L, Nagaraj K. Effect of Paneer and Cheese Consumption on Salivary Acidogenicity and Calcium Concentration: A Comparative Study. Oral Health Prev Dent 2018;16:169-74.
2. Sultan S, Telgi CR, Chaudhary S, Manuja N, Kaur H, Amit SA, et al. Effect of ACP-CPP chewing gum and natural chewable products on plaque pH, calcium and phosphate concentration. J Clin Diagn Res 2016;10:ZC13-7.
3. Kayalvizhi G, Subramaniyan RB, SuganyaG, Neerja R. Determining the efficacy of ocimum sanctum leaves extract on cariogenic properties of

streptococcus mutans An in vitro study. Eur J Pharm Med Res 2016;3:257-64.
4. Prabhakar A, Dodawad R, Os R. Evaluation of flow rate, pH, buffering capacity, calcium, total protein and total antioxidant levels of saliva in caries free and caries active children An In vivo study. Int J Clin Pediatr Dent 2009;2:9-12.
5. Tinanoff N, Palmer CA. Dietary determinants of dental caries and dietary recommendations for preschool children. J Public Health Dent 2000;60:197-206.
6. Moynihan P. Foods and factors that protect against dental caries. Nutr Bull 2000;25:281-28.
7. Preethi BP, Reshma D, Anand P. Evaluation of flow rate, pH, buffering capacity, calcium, total proteins and total antioxidant capacity levels of saliva in caries free and caries active children: An In vivo study. Indian J Clin Biochem 2010;25:425-8.
8. Kukreja BJ, Dodwad V. Herbal mouthwashes – A gift of nature. Int J Pharm Bio Sci 2012;3:46-52.
9. NagappanN, John J. Antimicrobial Efficacy of Herbal and Chlorhexidine Mouth rinse A systematic review. J Dent Med Sci 2012;2:5-10.
10. Jain I, Jain P, Bisht D, Sharma A, Srivastava B, Gupta N. Comparative evaluation of antibacterial efficacy of six indian plant extracts against streptococcus mutans. J Clin Diagn Res 2015;9:ZC50-3.
11. Botelho MA, Santos RA, Martins JG, Carvalho CO, Paz MC, Azenha C, et al. Efficacy of a mouthrinse based on leaves of the neem tree (Azadirachta indica) in the treatment of patients with chronic gingivitis: A doubleblind, randomized, controlled trial. J Med Plant Res. 2008;2:341–6.
12. Kochikar Pai R, Bhat SS, Salman A, Chandra J. Use of an extract of Indian sacred plant Ocimum sanctum as an anticariogenic agent: An in vitro study. Int J Clin Pediatr Dent 2015;8:99-101.
13. Edgar WM. The role of saliva in the control of pH changes in human dental plaque. Caries Res 1976;10:241-54.