Original Article

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

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Abstract

Background: Dental caries is an infectious disease affecting majority of children in the modern world. An acidogenic diet brings down plaque pH, leading to dental caries. However, certain foods bring about rapid reversal of plaque pH after an acidogenic challenge. *Azadirachta indica* and *Ocimum sanctum* leaves which are anticariogenic show promise in this regard.

Aim: The aim of this study was to evaluate the salivary pH reversal phenomenon by chewing *A. indica* and *O. sanctum* leaves after an acidogenic challenge.

Materials and Methods: Forty caries-free children were selected and randomly divided into two groups. The resting salivary pH and salivary pH after eating chocolate was measured using a pH meter at time intervals of 5, 30 and 60 min. The children were then instructed to chew *A. indica* and *O. sanctum* leaves after chocolate consumption, and salivary pH was again measured at 5, 30 and 60 min. The change in salivary pH was assessed.

Statistical Analysis: The data obtained were analyzed using Student's *t*-test (two tailed, dependent) to find the significance of the study parameters. The level of significance was set at $P < 0.05$.

Results: Chewing *A. indica* and *O. sanctum* leaves after chocolate consumption increased salivary pH to statistically significant values at 5, 30 and 60 min.

Conclusion: The observations of the study show that chewing of *A. indica* and *O. sanctum* leaves reversed the drop in salivary pH levels after an acidogenic challenge.

Keywords: *Azadirachta Indica*, dental caries, *Ocimum sanctum*, salivary pH

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INTRODUCTION

Dental caries is the most common chronic oral disease affecting children today.[1,2] Fermentable carbohydrates in the diet which retain in oral cavity for long time bring down the plaque pH, leading to dental caries. Hence, rapidly clearing cariogenic foods from the oral cavity by the consumption of protective foods is recommended.[3] Foods such as milk, cheese, unrefined plant foods and

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tea have shown to be caries protective.[4] The scientific community is constantly exploring for a locally available, economical, effective and culturally acceptable caries protective substance to help reduce dental caries.

Chewing of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) leaves is proved to be home remedies for the treatment of infections, tooth decay, bleeding and sore gums effectively.[5-7]

A. indica and *O. sanctum* leaves showed antibacterial activity against *Streptococcus mutans*, the microorganism responsible for dental caries.[8,9] *O. sanctum* leaf extract has also demonstrated antimicrobial activity against *S. mutans, Streptococcus sanguis, Streptococcus mitis* and *Lactobacillus acidophilus*.[10] It has also shown significant increase in plaque pH after 5 min and 30 min of chewing leaves.[11] Some authors have suggested that *O. sanctum* can be recommended as an anticaries mouthwash in children after further research.[12] Though both the herbs are anti-cariogenic, there are no studies available about their potential in reversing the cariogenic effects of other foods. The present study was conducted to evaluate the salivary pH reversal phenomenon by chewing these leaves after a cariogenic challenge.

MATERIALS AND METHODS

After obtaining ethical clearance for the study from the institutional ethical committee, forty caries-free children in the age group of 8–10 years reporting to the department of Pedodontics and Preventive Dentistry, Dayanandasagar College of Dental Sciences, Bengaluru, India, were randomly selected. Children with dental caries, systemic diseases, uncooperative behavior, and those unwilling to participate in the study were excluded.

The study participants were randomly divided into two groups – Group A (*A. indica* leaves) and Group B (*O. sanctum* leaves).

Oral prophylaxis was performed for all the participants 24 h prior to study. They were instructed to refrain from eating or drinking at least 2 h prior to the test. The participants were seated on the dental chair comfortably and asked to relax. About 1 mL of unstimulated saliva was collected in a sterile plastic container with the head bent forward, and the baseline pH of saliva was recorded. The children were then asked to eat a chocolate (Cadbury Diary Milk chocolate Bar – 13 g), and the saliva sample was collected at 5-min, 30-min and 60-min intervals to estimate the salivary pH following an acidogenic challenge. Later, the children were asked to eat another chocolate, and Group A children were asked to chew five to six fresh *A. indica* leaves, whereas Group B children chewed on five to six *O. sanctum* leaves for 1 min with 5 mL of water. After spitting out the leaves and thoroughly expectorating, the salivary samples were collected at 5-min, 30-min and 60-min intervals to estimate the salivary pH.

All the samples were sent immediately to the lab, and the pH of saliva was measured as soon as possible and not later than 30 min. Salivary pH was measured using a digital Digital pH meter (HI 2211 pH/ORP Meter, Hanna instruments, Rhode island, USA), calibrated with standard solutions of pH 4.0 and 7.0. The electrode was washed with distilled water and dried with absorbent paper after each analysis.

Statistical analysis using Student's *t*-test (two tailed, dependent) was done to compare the values within the groups. The $P < 0.05$ was fixed to be statistically significant.

RESULTS

In both Group A and Group B children, after consumption of chocolate, the salivary pH significantly dropped at 5 and 30 min and gradually returned to baseline values after approximately 60 min [Tables 1 and 2]. In Group A children, when *A. indica* leaves were chewed following the chocolate challenge, the pH increased to statistically significant values after 5, 30 and 60 min with value higher than baseline value at 60 min [Table 3 and Graph 1]. In Group B children, when *O. sanctum* leaves were chewed following the chocolate challenge, pH increased to statistically significant values after 5 and 30 min. The value at 60 min was slightly lesser than baseline value though not statistically significant [Table 4 and Graph 2]. When the groups were compared, a maximum rise in salivary pH was observed for *A. indica* leaves ($P < 0.05$), but the difference was statistically significant only at 30 min [Table 5 and Graph 3].

DISCUSSION

Most of the studies conducted to evaluate the acidogenic potential of substrates were based on the evaluation of plaque pH. However, our study focused on the reversal of salivary pH after an acidogenic challenge by locally available *A. indica* and *O. sanctum* leaves as it is established that the major factor controlling plaque pH is salivary pH.[13] In addition, salivary pH is easy to evaluate and educate children about the protective aspects of food during diet counseling.

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

	Minimum–maximum	Mean±SD	Difference	*t*	*P*
Baseline pH	6.47–7.83	7.20±0.37	-	-	-
After consuming chocolate					
5	5.30–7.26	6.64±0.51	0.551	11.701	<0.001*
30	6.21–7.36	6.90±0.35	0.297	7.126	<0.001*
60	6.40–7.78	7.16±0.40	0.030	1.289	0.213

*Statistically significant. SD: Standard deviation

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

	Minimum–maximum	Mean±SD	Difference	*t*	*P*
Baseline pH	6.11–8.52	7.29±0.59	-	-	-
After consuming chocolate (min)					
5	5.14–7.86	6.71±0.66	0.587	8.425	<0.001*
30	6.11–7.71	6.99±0.41	0.302	3.543	0.002*
60	6.13–7.98	7.22±0.50	0.071	1.956	0.066

*Statistically significant. SD: Standard deviation

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

	Minimum–maximum	Mean±SD	Difference	*t*	*P*
Base line pH	6.47–7.83	7.20±0.37	-	-	-
After consuming chocolates (min)			Comparison with values after consuming chocolates		
5	5.30–7.26	6.64±0.51	-	-	-
30	6.21–7.36	6.90±0.35	-	-	-
60	6.40–7.78	7.16±0.40	-	-	-
After chewing *Azadirachta indica* leaves following chocolate challenge (min)					
5	6.53–8.11	7.46±0.37	0.81	7.464	<0.001*
30	6.56–8.05	7.49±0.40	0.59	5.347	<0.001*
60	6.50–7.64	7.36±0.28	0.19	2.794	0.012*

*Statistically significant. SD: Standard deviation

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

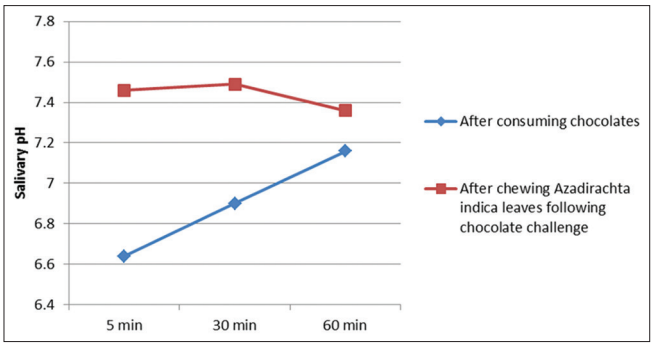
	Minimum-maximum	Mean±SD	Difference	*t*	*P*
Base line pH	6.11–8.52	7.29±0.59	-	-	-
After consuming chocolates (min)					
5	5.14–7.86	6.71±0.66	-	-	-
30	6.11–7.71	6.99±0.41	-	-	-
60	6.13–7.98	7.22±0.50	-	-	-
After chewing *Ocimum sanctum* (Tulsi) leaves following chocolate challenge (min)					
5	6.66–7.96	7.38±0.35	0.67	6.013	<0.001*
30	6.90–7.60	7.24±0.20	0.25	2.833	0.011*
60	6.12–7.96	7.25±0.46	0.026	0.967	0.347

*Statistically significant. SD: Standard deviation

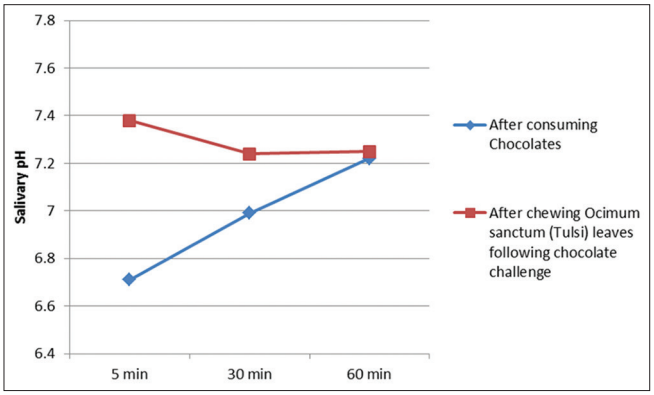
Table 5: Comparison of pH at different time intervals between Group A and B children

	Azadirachta indica (Neem) suppl	*Ocimum sanctum* (Tulsi) suppl	Total	*P*
Base line pH	7.20±0.37	7.29±0.59	7.24±0.4	0.530
After consuming chocolates (min)				
5	6.64±0.51	6.71±0.66	6.67±0.58	0.739
30	6.90±0.35	6.99±0.41	6.94±0.38	0.440
60	7.16±0.40	7.22±0.50	7.19±0.44	0.683
After chewing *Azadirachta indica*/ *Ocimum sanctum* leaves (min)				
5	7.46±0.37	7.38±0.35	7.42±0.35	0.511
30	7.49±0.40	7.24±0.20	7.37±0.34	0.023*
60	7.36±0.28	7.25±0.46	7.30±0.38	0.383

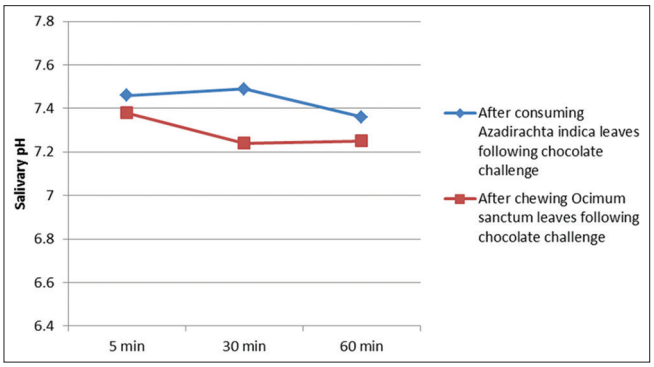
*Statistically significant



Graph 1: Assessment of salivary pH in Group A children after chewing *Azadirachta indica* leaves following a chocolate challenge



Graph 2: Assessment of salivary pH values in Group B children after chewing *Ocimum sanctum* leaves following a chocolate challenge



Graph 3: Comparison of salivary pH at different time intervals between Groups A and B children

The baseline salivary pH was recorded before giving the test foods to provide a value against which the change in pH could be compared. Normal salivary pH is in the range of 6.2–7.6. In this study, the pH ranged from 6.1 to 8.5, which confirms the previous reports, showing a subject-to-subject variation in salivary pH due to differences in caries susceptibility.[14]

In the present study, after the children consumed chocolate, there was a rapid drop in salivary pH, which is similar to that of Stephan's observations that sugar-containing foods cause a rapid drop in plaque (oral) pH.[15] Other studies conducted by Somaraj *et al*.[5] and Tayab *et al*.,[14] and Tahmassebi and Duggal,[16] showed similar fall in pH. The "Critical pH" for enamel is approximately 5.5, below which the enamel disintegrates.[17] The salivary pH recorded in the study was above the critical pH after the acidogenic challenge. This finding may be attributed to higher baseline pH of the children exhibiting good buffering capacity of the saliva and good oral hygiene with minimal plaque.[18] Furthermore, chocolate being a high-sugar food has a higher oral clearance as compared to high starch foods, which are more frequently consumed by children, leading to a lesser fall in pH.[19,20] The salivary pH values were estimated at 5, 30 and 60 min to record the time taken for the salivary pH to return to baseline values after an acidogenic challenge.[20,21] The study showed that the pH returned to baseline at 60 min, which is similar to other studies.[5,14]

Salivary pH is influenced by flow rate, duration of stimulation and calcium concentration; therefore, these children were instructed to chew *A. indica* and *O. sanctum* leaves for a minute to standardize the experimental conditions.[14] The salivary pH values were recorded after 5 min had elapsed because a minimum drop in pH after the chocolate challenge was seen at 5 min. In the present study, both *A. indica* and *O. sanctum* groups showed salivary pH reversal after a cariogenic challenge. There was a statistically significant rise in pH after chewing *A. indica* leaves after 5, 30 and 60 min. The *O. sanctum* group showed statistically significant rise in salivary pH after 5 and 30 min similar to other studies.[11] However, the exact mechanism of action is not known. It is shown that a food requiring a lot of chewing produces more copious salivary flow with an increased buffering capacity, thereby more effectively neutralizing plaque acids and aiding oral clearance of food debris.[21] This explanation could be attributed to the pH changes in this study also.

Diet counseling for prevention of dental caries involves recommendation for reduction in the frequency of carbohydrate consumption. Children frequently consume snacks containing carbohydrates. Though tooth brushing after sugar exposure is recommended, it may not be practical. Hence, chewing on caries protective foods after cariogenic challenge is a reasonable alternate.

CONCLUSION

Our study has shown that chewing of *A. indica* and *O. sanctum* leaves helps reversal of pH after a cariogenic challenge. This observation along with anticariogenic effect of these two herbs on oral health leads us to believe that

children can be encouraged to consume these leaves after carious exposure in-between meals. Further research with larger sample size is needed to support our findings.

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Conflicts of interest

There are no conflicts of interest.

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