



Evaluation of the Cariogenic Potential and Total Antioxidant Capacity of Saliva after the Consumption of Candies and Paneer: An In Vivo Study

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Abstract

Aim The present study was performed to assess and compare the changes in pH, calcium, phosphorus, alkaline phosphatase, and the total antioxidant capacity (TAC) of saliva after consuming candies, followed by rinsing with water and consumption of paneer.

Materials and Methods Baseline saliva of 5 mL was collected from 60 children and they were asked to chew on candies. After 10 minutes of intake of candies, 5 mL of saliva was collected. They were then divided into two groups of 30 each and test products were given. Group 1: rinse with water and group 2: chew 20 g of paneer and swallow. Saliva of % mL was collected after 10, 20, and, 30 minutes from the study groups. The saliva samples collected were assessed for pH, calcium, phosphorus, alkaline phosphatase, and TAC. The data were analyzed using the paired *t*-test and post hoc Tukey's test.

Results The pH, calcium, phosphorus, and alkaline phosphatase decreased from baseline values after consuming candies significantly ($p < 0.001$). These increased after rinsing with water and consumption of paneer which was statistically significant ($p < 0.001$). The TAC values increased significantly after consuming candies and decreased after rinsing with water and consumption of paneer which was substantial for both the groups.

Conclusion Consumption of candies created a cariogenic milieu. Both the study groups were effective in the reversal of the cariogenic potential. The consumption of paneer caused remineralizing properties and showed superior results than rinsing with water.

Keywords

- ▶ candies
- ▶ paneer
- ▶ pH
- ▶ calcium
- ▶ phosphorus
- ▶ alkaline phosphatase
- ▶ total antioxidant capacity

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Introduction

Dental caries, a common chronic childhood disease,¹ is initiated by consuming fermentable carbohydrates which are converted into organic acids by the cariogenic bacteria in dental plaque. These acids cause a fall in the salivary pH and result in demineralization by removing calcium and phosphate ions from the enamel subsurface.²

The 20th century has brought about a significant change in the dietary habits of children. Different types of chocolates are available in the market, and their frequent consumption has resulted in rampant caries at a very early age. The mean prevalence of dental caries in India for age group 5 and 12 years was 49% and for age 15 years was 60%,³ and those consuming substances containing sugar were at a 4.8 times higher risk.⁴ Cariogenicity in terms of pH and salivary clearance of commercially available chocolates has been studied^{5,6} and candies with increased sucrose content, higher frequency of intake, and delayed clearance were more cariogenic.^{5,6} There is a lack of data evaluating in vivo changes in the salivary parameters like calcium, phosphorus, alkaline phosphatase, and total antioxidant capacity (TAC) after consuming candies.

The cariogenic potential of food is related to the retention time of the acidic carbohydrates in the mouth, the role of saliva in oral clearance, the presence of remineralizing ions like calcium and phosphorus, and its buffering capacity.⁷ Research has shown that reduced calcium, phosphorus, and pH levels are associated with a higher prevalence of caries.^{8,9} Saliva has various protective antioxidant mechanisms which prevent formation of free radicals and repair oxidative damage caused by bacteria. Imbalance in levels of free radicals, reactive oxygen species, and antioxidants in saliva play an essential role in the onset and development of dental caries.¹⁰

Salivary calcium and phosphorus diffuse into the subsurface at the early stages of demineralization, causing a reversal.¹¹ Also, alkaline phosphatase, a nonspecific phosphomonoesterase, helps in producing free inorganic phosphate which provides ions for remineralization by common ion effect.¹² However, in children with high caries risk, due to repeated or increased exposure to cariogenic food, the salivary defense mechanism is not adequate and they require additional sources of these ions.

Diet counseling has been emphasized to prevent or reduce decay,¹³ with limitation of sugar intake being the key objective. However, lack of patient compliance renders this concept ineffective, creating a need for an alternative solution. Traditionally, it was thought that rinsing with water after the consumption of sucrose helped in oral clearance and elimination of the cariogenic challenge, its effect on various salivary parameters remains unknown.

There is also an increasing amount of data regarding the emerging role of milk and milk products in preventing dental caries and promoting remineralization.¹⁴⁻¹⁶ Apart from being natural, the benefits of milk are due to the presence of casein phosphopeptides, proteins, fats, vitamins, calcium, and phosphate.¹⁵ Lactoferrin and casein in milk and milk products inhibit the adherence of bacteria.¹⁷

Major emphasis has been placed on the anticariogenic properties of milk products, like paneer, which are economical and common constituents of the Indian diet. They cause saliva stimulation, enhancing their buffering capacity, and faster carbohydrate clearance.¹⁸⁻²¹ They are also rich in proteins like casein, calcium, and phosphorus. Paneer or Indian cottage cheese is unsweetened, an unripened form of cheese with lesser content of fatty acids and preservatives than commercial cheese.²² However, there are limited data on the antioxidant properties of dairy products like paneer. Hence, this study was undertaken to assess the efficacy of this dairy product to counteract the cariogenic challenge of sweetened candies.

Materials and Methods

A comparative study was conducted at Attavar Balakrishna Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangaluru, Karnataka, India. Sixty children between the age group of 5 to 12 years who reported for dental treatment to the Outpatient Department of Pediatric and Preventive Dentistry were included in the study. The sample size estimation was calculated using the following formula:

$$n = \frac{2(Z_{\alpha} + Z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

Normal children, who do not have any systemic or metabolic disease and special health care needs with decayed missing filled teeth (DMFT)/deft 3 to 5, were included in the study according to the World Health Organization (WHO) criteria.

Caries-free children, children with teeth having enamel defects, children on medication due to any systemic and metabolic diseases, and children with special health care needs were excluded from the study. In addition, clearance from the Institutional Ethics Committee (reference no.: ABSM/EC/75/2013) was obtained.

According to the WHO criteria, patients were screened using mouth mirror and probe, and children with active caries were selected for the study. In addition, the study was explained to the parents and the patients, and written consent from parents/guardians and verbal assent of the child was obtained.

Oral prophylaxis was done 24 hours before the study to maintain standardization. Patients were asked to refrain from brushing on the day of salivary sample collection and eating or drinking 2 hours before the examination. In addition, 5 mL of baseline unstimulated saliva was collected by making the child sit in "Coachman's position" at the edge of the chair and passively drooling saliva in sterilized plastic containers. This saliva sample was analyzed for pH, calcium, phosphorus, alkaline phosphatase, and TAC.

All Children were given one sweetened candy, and they were asked to suck on it till they finished it. The children took approximately 5 minutes to complete the entire candy. Saliva sample of 5 mL was collected after 10 minutes of candy consumption by the method as mentioned above.

The patients were then randomly divided into two groups of 30 each. Group 1: rinse with water and group 2: chew on 20-g paneer and then swallow it.

Saliva of 5 mL sample was then collected by the same method at 10-, 20-, and 30-minute intervals after the consumption of water and paneer. The patients were later asked to brush thoroughly to remove any candy particles adhering to the tooth. The salivary samples were stored at -20°C till the biochemical analysis was performed. All the collected saliva samples were centrifuged at 3,000 rpm for 10 minutes, and the supernatant was used for biochemical analysis. Supernatant saliva was analyzed for pH, calcium, phosphorus, alkaline phosphatase, and TAC.

The biochemical methods used to analyze salivary pH, calcium, phosphorous, and alkaline phosphatase were digital pH meter, modified o-Cresolphthalein complexone (OCPC) method, phosphomolybdate method, and Deutsche Gesellschaft Fur Klinische Chemie – Scandinavian Society of Clinical Chemistry (DGKC-SCE) method, respectively. TAC was measured by phosphomolybdenum method and analyzed by spectrophotometer.

Statistical Analysis

Paired *t*-test was used to analyze the changes in salivary parameters at baseline; 10 minutes after the consumption of candies; and 10, 20, and 30 minutes after water and paneer. The level of significance was set at $p < 0.05$. In addition, evaluation of intergroup changes in the salivary parameters from candies and at 10-, 20-, and 30-minute intervals after the intake of water and paneer was analyzed by post hoc Tukey's test.

Results

pH

The mean baseline pH of saliva decreased after consuming candies and increased steadily in group I from 10, 20, to

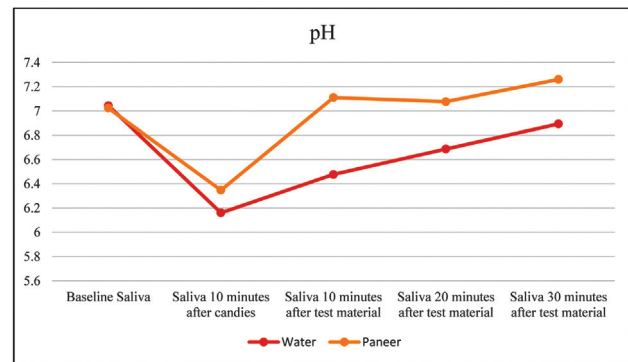


Fig. 1 The pH levels in saliva at baseline, after consumption of candies followed by water and paneer at different time intervals.

30 minutes which was highly significant ($p < 0.001$). However, the final mean pH after 30 minutes was lower than the baseline. This difference was highly significant ($p < 0.001$; ►Table 1; ►Fig. 1).

The reduction in the mean baseline pH after consumption of candies was increased in group II at 10 to 30 minutes, with the highest value at 30 minutes. Even though a slight drop in pH was noted after 20 minutes of consumption of paneer, all values stayed more elevated than the levels seen after consumption of candies, significantly ($p < 0.001$). The final value achieved at the end of 30 minutes after paneer consumption was higher than the baseline pH values and was statistically significant ($p = 0.004$; ►Table 2; ►Fig. 1).

Intergroup comparison of the changes in salivary pH from baseline after rinsing with water and paneer consumption have shown that increase in the pH value seen after

Table 1 Salivary pH at baseline, after the consumption of candies and after rinsing with water

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (salivary pH)	Mean	n	Standard deviation	Mean	Standard deviation		
1	Baseline	7.043333	30	0.3136914	0.883333	0.296047	16.343	<0.001 ^a
	10 minutes after candies	6.160000	30	0.3420022				
2	10 minutes after candies	6.160000	30	0.3420022	-0.31667	0.258755	-6.703	<0.001 ^a
	10 minutes after water	6.476667	30	0.3757139				
3	10 minutes after candies	6.160000	30	0.3420022	-0.52667	0.3237	-8.912	<0.001 ^a
	20 minutes after water	6.686667	30	0.3785028				
4	10 minutes after candies	6.160000	30	0.3420022	-0.73333	0.272072	-14.763	<0.001 ^a
	30 minutes after water	6.89333333	30	0.297035158				
5	10 minutes after water	6.476667	30	0.3757139	-0.21	0.22796	-5.046	<0.001 ^a
	20 minutes after water	6.686667	30	0.3785028				
6	10 minutes after water	6.476667	30	0.3757139	-0.41667	0.226035	-10.097	<0.001 ^a
	30 minutes after water	6.89333333	30	0.297035158				
7	20 minutes after water	6.686667	30	0.3785028	-0.20667	0.214851	-5.269	<0.001 ^a
	30 minutes after water	6.89333333	30	0.297035158				
8	Baseline	7.043333	30	0.3136914	0.15	0.171705	4.785	<0.001 ^a
	30 minutes after water	6.89333333	30	0.297035158				

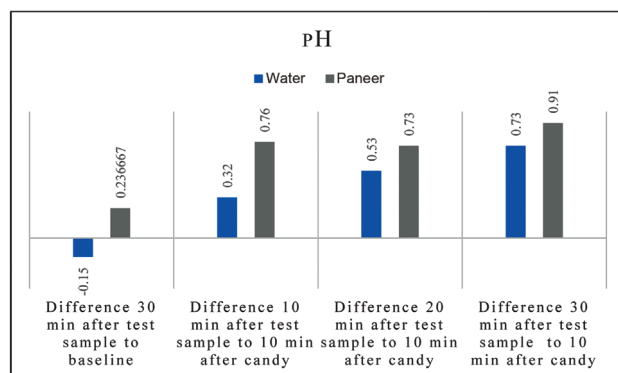
^a $p < 0.001$: very highly significant.

Table 2 Salivary pH at baseline, after the consumption of candies and after the consumption of paneer (20 g)

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (salivary pH)	Mean	n	Standard deviation	Mean	Standard deviation		
1	Baseline	7.023333	30	0.5015035	0.676667	0.507654	7.301	<0.001 ^c
	10 minutes after candies	6.346667	30	0.4761435				
2	10 minutes after candies	6.346667	30	0.4761435	-0.76333	0.567805	-7.363	<0.001 ^c
	10 minutes after paneer	7.110000	30	0.5862505				
3	10 minutes after candies	6.346667	30	0.4761435	-0.73	0.463978	-8.618	<0.001 ^c
	20 minutes after paneer	7.076667	30	0.4264394				
4	10 minutes after candies	6.346667	30	0.4761435	-0.91333	0.459185	-10.894	<0.001 ^c
	30 minute after paneer	7.2600000	30	0.402235135				
5	10 minute after paneer	7.110000	30	0.5862505	0.033333	0.392458	0.465	0.645 ^a
	20 minutes after paneer	7.076667	30	0.4264394				
6	10 minutes after paneer	7.110000	30	0.5862505	-0.15	0.462191	-1.778	0.086 ^a
	30 minutes after paneer	7.2600000	30	0.402235135				
7	20 minutes after paneer	7.076667	30	0.4264394	-0.18333	0.304091	-3.302	0.003 ^b
	30 minutes after paneer	7.2600000	30	0.402235135				
8	Baseline	7.023333	30	0.5015035	-0.23667	0.40978	-3.163	0.004 ^b
	30 minutes after paneer	7.2600000	30	0.402235135				

^a $p > 0.05$: not significant.^b $p < 0.05$: significant.^c $p < 0.001$: very highly significant**Table 3** Intergroup comparison of changes in salivary pH from baseline after rinsing with water and consumption of paneer: post hoc Tukey's test

Parameter		I group	J group	Mean difference (I-J)	Standard error	p-Value
pH	Difference 30 minutes after test sample to baseline	Water	Paneer	-0.39	0.09	5.5E-05
	Difference 10 minutes after test sample to 10 minute after candy	Water	Paneer	-0.45	0.11	0.000386
	Difference 20 minutes after test sample to 10 minute after candy	Water	Paneer	-0.20	0.11	0.182512
	Difference 30 minutes after test sample to 10 minute after candy	Water	Paneer	-0.18	0.10	0.186158

Note: $E = 10^x$.**Fig. 2** Intergroup comparison of changes in salivary pH from baseline after rinsing with water and consumption of paneer.

10 minutes of paneer consumption was significantly higher than water. The comparison between the changes in pH in water and paneer has shown that the pH in the paneer group achieved after 30 minutes was significantly higher than the baseline compared with the water group (\rightarrow Table 3; \rightarrow Fig. 2).

Calcium

A decline in the mean calcium values from baseline after the consumption of candies was highly significant ($p < 0.001$). The calcium values increased after rinsing with water from 10, 20, to 30 minutes, and the difference was highly significant ($p < 0.001$). After 30 minutes of rinsing with water, the level was slightly above the baseline, and the difference was not significant. (\rightarrow Table 4; \rightarrow Fig. 3).

Table 4 Calcium level in saliva at baseline, after the consumption of candies and after rinsing with water

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (salivary calcium)	Mean (mg/dL)	n	Standard deviation	Mean (mg/dL)	Standard deviation		
1	Baseline	9.807017	30	1.1327041	2.211558	0.980069	12.36	<0.001 ^c
	10 minutes after candies	7.595459	30	1.1860537				
2	10 minutes after candies	7.595459	30	1.1860537	-1.25681	0.834719	-8.247	<0.001 ^c
	10 minutes after water	8.852269	30	1.3128995				
3	10 minutes after candies	7.595459	30	1.1860537	-1.74994	0.968391	-9.898	<0.001 ^c
	20 minutes after water	9.345394	30	1.0713825				
4	10 minutes after candies	7.595459	30	1.1860537	-2.39747	0.926998	-14.166	<0.001 ^c
	30 minutes after water	9.99292667	30	1.1206955				
5	10 minutes after water	8.852269	30	1.3128995	-0.49313	0.920182	-2.935	0.006 ^b
	20 minutes after water	9.345394	30	1.0713825				
6	10 minutes after water	8.852269	30	1.3128995	-1.14066	0.901694	-6.929	<0.001 ^c
	30 minutes after water	9.99292667	30	1.1206955				
7	20 minutes after water	9.345394	30	1.0713825	-0.64753	0.680506	-5.212	<0.001 ^c
	30 minutes after water	9.99292667	30	1.1206955				
8	Baseline	9.807017	30	1.1327041	-0.18591	0.665972	-1.529	0.137 ^a
	30 minutes after water	9.99292667	30	1.1206955				

^a $p > 0.05$: not significant.

^b $p < 0.05$: significant.

^c $p < 0.001$: very highly significant.

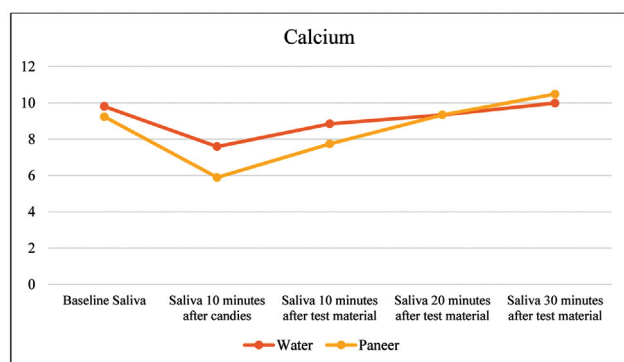


Fig. 3 Changes in the salivary calcium levels from baseline, after consumption of candies followed by water and paneer at different time intervals.

The mean baseline drop in the calcium level 10 minutes after the consumption of candies was increased after the intake of 20 g of paneer after 10, 20, and 30 minutes, significantly, and reached the highest value after 30 minutes. This increase was higher than baseline values and those seen after consuming candies and was statistically highly significant ($p < 0.001$; ►Table 5; ►Fig. 3).

Intergroup comparison of changes in salivary calcium from baseline after rinsing with water and after the consumption of paneer that has shown a rise in calcium level after 10, 20, and 30 minutes of test sample was higher for the paneer group as compared with water group, and the difference was statistically significant. The difference in the mean

calcium values after 30 minutes of paneer from baseline was significantly higher compared with the water group (►Table 6; ►Fig. 4).

Phosphorus

The baseline phosphorus value decreased after consumption of candies significantly ($p < 0.001$) and increased after rinsing with water from 10 minutes, with the highest value seen after 20 minutes significantly ($p < 0.001$). Though the final value at 30 minutes after rinsing with water was markedly higher than that seen after candies ($p < 0.001$), it was lesser than the baseline value with the significance of $p = 0.043$ (►Table 7; ►Fig. 5).

The drop in mean baseline phosphorus levels in saliva after the consumption of candies was significantly increased ($p < 0.001$) 10 minutes after the consumption of paneer. The phosphorus value declined gradually after 20 and 30 minutes of consumption of paneer but remained above the baseline value. The difference between phosphorus level at baseline and 30 minutes after chewing paneer was highly, statistically significant ($p < 0.001$; ►Table 8; ►Fig. 5).

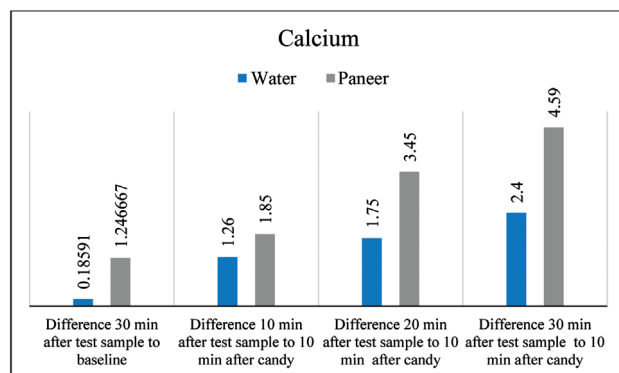
Intergroup comparison of changes in salivary phosphorus from baseline after rinsing with water and consumption of paneer has shown a higher rise in the phosphorus values in paneer group compared with the water 10 minutes after the consumption of paneer, and the difference was statistically significant. Paneer and water groups showed similar phosphorus values after 20 and 30 minutes. The difference between the values achieved after 30 minutes from the

Table 5 Calcium level in saliva at baseline, after the consumption of candies and after the consumption of paneer (20 g)

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (salivary calcium)	Mean (mg/dL)	n	Standard deviation	Mean (mg/dL)	Standard deviation		
1	Baseline	9.239000	30	1.1092023	3.347367	1.689329	10.853	<0.001 ^b
	10 minutes after candies	5.891633	30	1.5165071				
2	10 minutes after candies	5.891633	30	1.5165071	-1.85209	1.978874	-5.126	<0.001 ^b
	10 minutes after paneer	7.743727	30	1.7982893				
3	10 minutes after candies	5.891633	30	1.5165071	-3.45177	2.188084	-8.64	<0.001 ^b
	20 minutes after paneer	9.343400	30	1.6985676				
4	10 minutes after candies	5.891633	30	1.5165071	-4.59403	1.643682	-15.309	<0.001 ^b
	30 minutes after paneer	10.48566667	30	1.321948284				
5	10 minutes after paneer	7.743727	30	1.7982893	-1.59967	2.374102	-3.691	0.001 ^a
	20 minutes after paneer	9.343400	30	1.6985676				
6	10 minutes after paneer	7.743727	30	1.7982893	-2.74194	2.421186	-6.203	<0.001 ^b
	30 minutes after paneer	10.48566667	30	1.321948284				
7	20 minutes after paneer	9.343400	30	1.6985676	-1.14227	1.780574	-3.514	0.001 ^a
	30 minutes after paneer	10.48566667	30	1.321948284				
8	Baseline	9.239000	30	1.1092023	-1.24667	1.250632	-5.46	<0.001 ^b
	30 minutes after paneer	10.48566667	30	1.321948284				

^a $p < 0.01$: highly significant.^b $p < 0.001$: very highly significant.**Table 6** Intergroup comparison of changes in salivary calcium from baseline after rinsing with water and consumption of paneer (post hoc Tukey's test)

Parameter		I group	J group	Mean difference (I-J)	Standard error	p-Value
Calcium	Difference between 30 minutes after test sample to baseline	Water	Paneer	-1.06	0.26	0.000238
	Difference between 10 minutes after test sample to 10 minute after candy	Water	Paneer	-0.60	0.37	0.245738
	Difference between 20 minutes after test sample to 10 minute after candy	Water	Paneer	-1.70	0.41	0.000212
	Difference between 30 minutes after test sample to 10 minute after candy	Water	Paneer	-2.20	0.35	4.03E-08

Note: E = 10^x.**Fig. 4** Intergroup comparison of changes in salivary calcium from baseline after rinsing with water and consumption of paneer.

baseline of the test sample was significantly higher in the paneer than in the water group (►Table 9; ►Fig. 6).

Alkaline Phosphatase

The mean baseline alkaline phosphatase level decreased after intake of candies which was highly significant ($p < 0.001$). This value increased after rinsing with water at 10 ($p = 0.001$), 20, and 30 minutes ($p < 0.001$) significantly. The highest value noted was 30 minutes after rinsing with water and was similar to baseline (►Table 10; ►Fig. 7).

Reduction in the mean alkaline phosphatase level of saliva after consuming candies showed a gradual increase after chewing paneer from 10 to 30 minutes, and the increase was statistically significant ($p < 0.001$). The highest value noted

Table 7 Phosphorus level in saliva at baseline, after the consumption of candies and after rinsing with water

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (salivary phosphorus)	Mean (mM/L)	n	standard deviation	Mean (mM/L)	Standard deviation		
1	Baseline	3.022735	30	1.1883085	1.64359	0.866504	10.389	<0.001 ^c
	10 minutes after candies	1.379146	30	0.8992194				
2	10 minutes after candies	1.379146	30	0.8992194	-1.33733	1.167673	-6.273	<0.001 ^c
	10 minutes after water	2.716473	30	1.5984194				
3	10 minutes after candies	1.379146	30	0.8992194	-1.58625	0.947314	-9.171	<0.001 ^c
	20 minutes after water	2.965391	30	1.2216327				
4	10 minutes after candies	1.379146	30	0.8992194	-1.47992	0.799888	-10.134	<0.001 ^c
	30 minutes after water	2.85906100	30	1.118400332				
5	10 minutes after water	2.716473	30	1.5984194	-0.24892	0.930019	-1.466	0.153 ^a
	20 minutes after water	2.965391	30	1.2216327				
6	10 minutes after water	2.716473	30	1.5984194	-0.14259	1.223582	-0.638	0.528 ^a
	30 minutes after water	2.85906100	30	1.118400332				
7	20 minutes after water	2.965391	30	1.2216327	0.10633	0.81816	0.712	0.482 ^a
	30 minutes after water	2.85906100	30	1.118400332				
8	Baseline	3.022735	30	1.1883085	0.163674	0.423943	2.115	0.043 ^b
	30 minutes after water	2.85906100	30	1.118400332				

^a $p > 0.05$: not significant.

^b $p < 0.05$: significant.

^c $p < 0.001$: very highly significant.

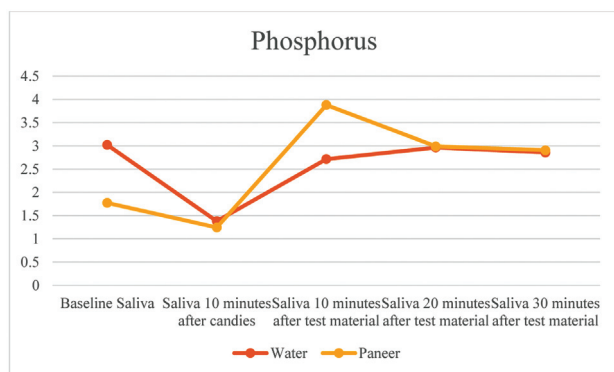


Fig. 5 Changes in the salivary phosphorus levels from baseline, after consumption of candies followed by water and paneer at different time intervals.

was at 30 minutes after chewing paneer, and it was significantly higher than the baseline ($p < 0.001$; ► **Table 11**; ► **Fig. 7**).

Salivary alkaline phosphatase from baseline after rinsing with water and 10 minutes after the consumption of paneer showed a significant increase in the values after candies which were comparable to water group. The rise in alkaline phosphatase seen after 20 and 30 minutes of consumption of the test material was similar in both groups. The difference between baseline level and after 30 minutes of the test material was significantly higher for the paneer group than the water group. The difference between the values achieved after 30 minutes from baseline of test sample was significantly higher in paneer group than the water group (► **Table 12**; ► **Fig. 8**).

Total Antioxidant Capacity

There was an increase in the TAC from the baseline value 10 minutes after consuming candies and was statistically significant ($p < 0.001$). This value decreased after rinsing with water significantly at 10 ($p = 0.003$), 20 ($p = 0.001$), and 30 minutes ($p < 0.001$), with the most negligible value noted after 30 minutes. The final value achieved 30 minutes after rinsing with water was similar to the baseline value (► **Table 13**; ► **Fig. 9**).

The increase in mean baseline TAC value after the consumption of candies was decreased gradually after consumption of paneer from 10 to 30 minutes; however, the final values observed after 30 minutes were still higher than baseline, and these differences were statistically not significant (► **Table 14**; ► **Fig. 9**).

The difference in TAC values after consuming candies and 10, 20, and 30 minutes of test materials were significantly higher in the paneer group than the water group. The difference between the TAC of saliva at baseline and 30 minutes after consumption of test material was higher for the paneer group than water, but the difference was not statistically significant (► **Table 15**; ► **Fig. 10**).

Discussion

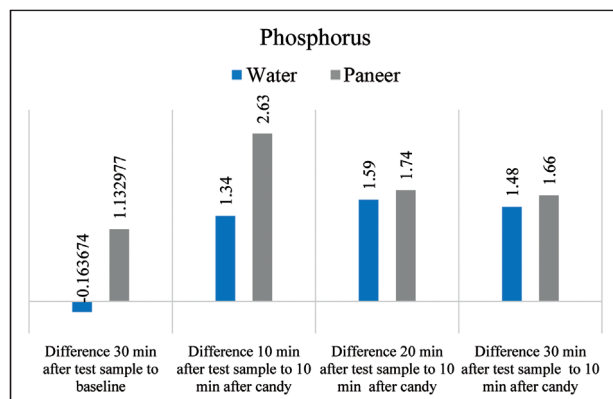
Key factors in determining dietary cariogenic, cariostatic, and anticariogenic properties are the food form and texture, frequency of fermentable carbohydrate consumption, retention time, nutrient composition, and the potential of the food

Table 8 Phosphorus level in saliva at baseline, after the consumption of candies and after the consumption of paneer (20 g)

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (salivary phosphorus)	Mean (mM/L)	n	Standard deviation	Mean (mM/L)	Standard deviation		
1	Baseline	1.773733	30	0.4533707	0.528297	0.765219	3.781	0.001 ^c
	10 minutes after candies	1.245437	30	0.7030305				
2	10 minutes after candies	1.245437	30	0.7030305	-2.63403	1.28481	-11.229	<0.001 ^d
	10 minutes after paneer	3.879467	30	1.4379901				
3	10 minutes after candies	1.245437	30	0.7030305	-1.74426	1.431895	-6.672	<0.001 ^d
	20 minutes after paneer	2.989700	30	1.0576180				
4	10 minutes after candies	1.245437	30	0.7030305	-1.66127	1.067084	-8.527	<0.001 ^d
	30 minutes after paneer	2.90671000	30	0.807100946				
5	10 minutes after paneer	3.879467	30	1.4379901	0.889767	1.84942	2.635	0.013 ^b
	20 minutes after paneer	2.989700	30	1.0576180				
6	10 minutes after paneer	3.879467	30	1.4379901	0.972757	1.616166	3.297	0.003 ^b
	30 minutes after paneer	2.90671000	30	0.807100946				
7	20 minutes after paneer	2.989700	30	1.0576180	0.08299	1.198061	0.379	0.707 ^a
	30 minutes after paneer	2.90671000	30	0.807100946				
8	Baseline	1.773733	30	0.4533707	-1.13298	0.983994	-6.307	<0.001 ^d
	30 minutes after paneer	2.90671000	30	0.807100946				

^a $p > 0.05$: not significant.^b $p < 0.05$: significant.^c $p < 0.01$: highly significant.^d $p < 0.001$: very highly significant.**Table 9** Intergroup comparison of changes in salivary phosphorus from baseline after rinsing with water and consumption of paneer (post hoc Tukey's test)

Parameter	I group	J group	Mean difference (I-J)	Standard error	p-Value	
Phosphorous	Difference between 30 minutes after test sample to baseline	Water	Paneer	-1.30	0.22	3.49E-07
	Difference between 10 minutes after test sample to 10 minutes after candy	Water	Paneer	-1.30	0.29	5.8E-05
	Difference between 20 minutes after test sample to 10 minutes after candy	Water	Paneer	-0.16	0.33	0.882632
	Difference between 30 minutes after test sample to 10 minute after candy	Water	Paneer	-0.18	0.27	0.777574

Note: E = 10^x.**Fig. 6** Intergroup comparison of changes in salivary phosphorus from baseline after rinsing with water and consumption of paneer.

to stimulate saliva and different combinations of food. The cariogenic potential of sweetened candies has already been established and hence we have chosen them as the demineralizing agent in this study.

Milk and dairy products form an essential part of the daily diet. They are not only natural but also act as precursors for various bioactive substances. They have also been recommended because they are a rich source of multiple proteins, potassium, vitamin D, lactoferrin, lysozyme, fats, and minerals like calcium and phosphorus. There is a considerable amount of data available on the protective effect of milk and milk products against dental caries.¹⁵⁻¹⁷

Research suggests that the beneficial role of milk and related products can be attributed to its high buffering capacity and other components like high calcium,

Table 10 Alkaline phosphatase level in saliva at baseline, after the consumption of candies and after rinsing with water

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (alkaline phosphatase)	Mean (U/L)	n	Standard deviation	Mean (U/L)	Standard deviation		
1	Baseline	47.998133	30	35.3033127	21.71813	30.30231	3.926	<0.001 ^c
	10 minutes after candies	26.280000	30	16.9392607				
2	10 minutes after candies	26.280000	30	16.9392607	-18.368	27.55319	-3.651	0.001 ^b
	10 minutes after water	44.648000	30	32.8716269				
3	10 minutes after candies	26.280000	30	16.9392607	-17.6657	21.31189	-4.54	<0.001 ^c
	20 minutes after water	43.945667	30	24.1040071				
4	10 minutes after candies	26.280000	30	16.9392607	-22.489	26.15324	-4.71	<0.001 ^c
	30 minutes after water	48.76900000	30	25.606007559				
5	10 minutes after water	44.648000	30	32.8716269	0.702333	23.49903	0.164	0.871 ^a
	20 minutes after water	43.945667	30	24.1040071				
6	10 minutes after water	44.648000	30	32.8716269	-4.121	31.31382	-0.721	0.477 ^a
	30 minutes after water	48.76900000	30	25.606007559				
7	20 minutes after water	43.945667	30	24.1040071	-4.82333	22.49303	-1.175	0.25 ^a
	30 minutes after water	48.76900000	30	25.606007559				
8	Baseline	47.998133	30	35.3033127	-0.77087	28.09139	-0.15	0.882 ^a
	30 minutes after water	48.76900000	30	25.606007559				

^a $p > 0.05$: not significant.

^b $p < 0.01$: highly significant.

^c $p < 0.001$: very highly significant.

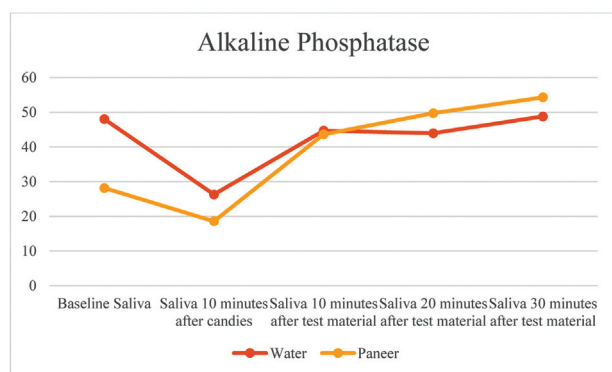


Fig 7 Changes in the salivary alkaline phosphatase levels from baseline, after consumption of candies followed by water and paneer at different time intervals.

phosphorus, lactoperoxidase, and casein. They have also been implicated to play a role in the prevention of bacterial attachment and biofilm formation by mechanical barrier formation or by inhibiting the bacterial glucosyltransferase enzyme.^{15,23} Lactoperoxidase and lysozyme in milk products inhibit the metabolism of *Streptococcus mutans*.¹⁵ Casein phosphopeptide (CPP), a fraction of casein present in dairy products, adsorbs to the enamel surface and impedes demineralization. It stabilizes amorphous calcium phosphate (ACP) which helps diffuse free calcium into the tooth.²⁴

Commercially available remineralization technologies utilizing CPP-ACP complex have been proved as an adjunct to fluoride therapy in the remineralization of early carious

lesions.²⁵ We have used paneer in our study which is rich in casein, calcium, and phosphorus and has a similar mechanism of action. Paneer is natural, economical and can be homemade. It can be consumed by all age groups and patients with lactose intolerance without any health hazards. It has also been recommended for growing children due to the high content of various minerals, like calcium, phosphorus, magnesium, vitamin D, and others, which helps in healthy teeth, as well as bone formation.²⁶

The integrity of the oral cavity is maintained by saliva by mechanical cleansing. It aids oral clearance; has antioxidants to counteract oxidative stress; buffers the harmful effects of acids; provides ions, like iron, magnesium, phosphorus, calcium, fluoride, and others, for remineralization; and has various antibacterial properties.²⁷ The delicate equilibrium of demineralization and remineralization to which the teeth are subjected to dwells on the pH, calcium, phosphorus, alkaline phosphatase, and TAC of saliva. Thus in our study, we evaluated changes in these factors after a cariogenic challenge and its reversal with dairy products.

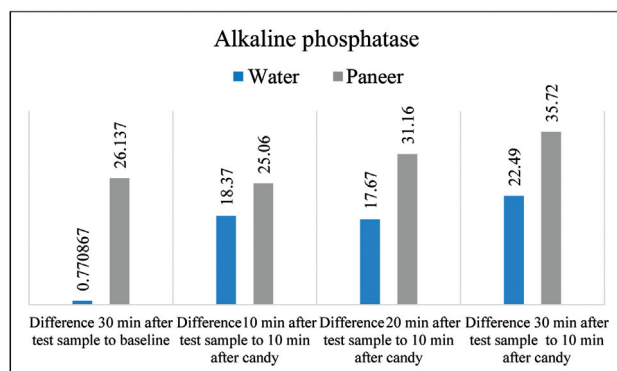
In our study, consumption of candies produced a cariogenic challenge and it was reflected as a sharp decline in the salivary pH 10 minutes after the consumption of candies which is similar to the findings of Tayab et al.²² and de Sousa et al.²⁸ This fall in pH is due to the production of organic acids, like lactic, acetic, propionic, butyric, and formic acids, by the plaque bacteria after the exposure to fermentable carbohydrates present in candies and is following Stephan's curve.

Table 11 Alkaline phosphatase level in saliva at baseline, after the consumption of candies and after the consumption of paneer (20 g)

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (alkaline phosphatase)	Mean (U/L)	n	Standard deviation	Mean (U/L)	Standard deviation		
1	Baseline	28.136333	30	11.7879253	9.585267	12.72702	4.125	<0.001 ^c
	10 minutes after candies	18.551067	30	10.1201537				
2	10 minutes after candies	18.551067	30	10.1201537	-25.0596	27.74408	-4.947	<0.001 ^c
	10 minutes after paneer	43.610667	30	29.7177747				
3	10 minutes after candies	18.551067	30	10.1201537	-31.1643	34.50689	-4.947	<0.001 ^c
	20 minutes after paneer	49.715333	30	34.6158926				
4	10 minutes after candies	18.551067	30	10.1201537	-35.7223	22.32262	-8.765	<0.001 ^c
	30 minutes after paneer	54.2733333	30	19.78312662				
5	10 minutes after paneer	43.610667	30	29.7177747	-6.10467	38.23993	-0.874	0.389 ^a
	20 minutes after paneer	49.715333	30	34.6158926				
6	10 minutes after paneer	43.610667	30	29.7177747	-10.6627	28.38821	-2.057	0.049 ^b
	30 minutes after paneer	54.2733333	30	19.78312662				
7	20 minutes after paneer	49.715333	30	34.6158926	-4.558	38.41134	-0.65	0.521 ^a
	30 minutes after paneer	54.2733333	30	19.78312662				
8	Baseline	28.136333	30	11.7879253	-26.137	22.22304	-6.442	<0.001 ^c
	30 minutes after paneer	54.2733333	30	19.78312662				

^ap > 0.05: not significant.^bp < 0.05: significant.^cp < 0.001: very highly significant.**Table 12** Intergroup comparison of changes in salivary alkaline phosphatase from baseline after rinsing with water and consumption of paneer (post hoc Tukey's test)

Parameter		I group	J group	Mean difference (I-J)	Standard error	p-Value
Alkaline phosphatase	Difference between 30 s after test sample to baseline	Water	Paneer	-25.37	6.35	0.000396
	Difference between 10 minutes after test sample to 10 minute after candy	Water	Paneer	-6.69	6.63	0.573397
	Difference between 20 minutes after test sample to 10 minute after candy	Water	Paneer	-13.50	9.77	0.354843
	Difference between 30 minutes after test sample to 10 minute after candy	Water	Paneer	-13.23	5.99	0.075505

**Fig. 8** Intergroup comparison of changes in salivary alkaline phosphatase from baseline after rinsing with water and consumption of paneer.

The rise in pH after rinsing with water can be attributed to the mechanical clearance of the cariogenic challenge and saliva stimulation. The significant rise in pH after consumption of paneer from 10 to 30 minutes was significantly higher than the baseline pH. This observation was similar to that by Sharma et al,¹⁶ Sönmez and Aras,¹⁹ Tayab et al,²² Higham and Edgar,²⁹ and Somaraj et al³⁰ and who demonstrated a pH reversal when paneer was eaten after sugar exposure. This shows the prolonged anticariogenic effects of paneer by acting as sialogogues, increasing the buffering capacity of saliva,³¹ and enhancing the carbohydrate clearance due to their physical form and texture. Casein, lactoperoxidase, and lysozyme in milk products inhibit adherence and metabolism of *S. mutans* which further reduces the acids

Table 13 TAC level in saliva at baseline, after the consumption of candies and after rinsing with water

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (TAC)	Mean (mM/L)	n	Standard deviation	Mean (mM/L)	Standard deviation		
1	Baseline	0.984111	30	0.4907979	-0.96918	1.292882	-4.106	<0.001 ^d
	10 minutes after candies	1.953290	30	1.1514248				
2	10 minutes after candies	1.953290	30	1.1514248	0.656449	1.120536	3.209	0.003 ^b
	10 minutes after water	1.296842	30	0.4914402				
3	10 minutes after candies	1.953290	30	1.1514248	1.035572	1.452751	3.904	0.001 ^c
	20 minutes after water	0.917718	30	0.4378874				
4	10 minutes after candies	1.953290	30	1.1514248	1.095358	1.295519	4.631	<0.001 ^d
	30 minutes after water	0.85793244	30	0.359065346				
5	10 minutes after water	1.296842	30	0.4914402	0.379124	0.68005	3.054	0.005 ^b
	20 minutes after water	0.917718	30	0.4378874				
6	10 minutes after water	1.296842	30	0.4914402	0.438909	0.632879	3.799	0.001 ^c
	30 minutes after water	0.85793244	30	0.359065346				
7	20 minutes after water	0.917718	30	0.4378874	0.059786	0.596044	0.549	0.587 ^a
	30 minutes after water	0.85793244	30	0.359065346				
8	Baseline	0.984111	30	0.4907979	0.126178	0.641432	1.077	0.29 ^a
	30 minutes after water	0.85793244	30	0.359065346				

^a $p > 0.05$: not significant.

^b $p < 0.05$: significant.

^c $p < 0.01$: highly significant.

^d $p < 0.001$: very highly significant.

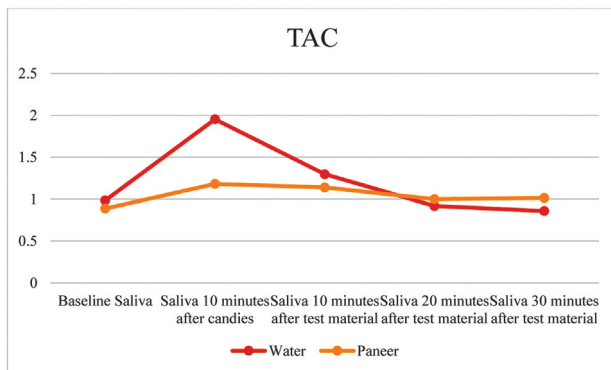


Fig. 9 Changes in the salivary TAC from baseline, after consumption of candies followed by water and paneer at different time intervals. TAC, total antioxidant capacity.

produced.³² CPP released by proteolysis of dairy products increases saliva saturation for calcium and phosphorus, increasing the pH.

The significant drop in calcium and phosphorus levels in saliva 10 minutes after consuming candies demonstrate their ability to cause demineralization. These ions may be utilized from the saliva to maintain the ionic gradient. Also, lower calcium and phosphorus levels have been seen in previous studies in children with high caries incidence.^{8,33}

After the cariogenic challenge, the consumption of paneer showed a significant rise in calcium and phosphorus values in our study after 10 minutes itself which could be due to the

higher levels of calcium and phosphorus in paneer. This correlates with the study by Ravishankar et al.³² and Moynihan et al.¹⁸ Kashket and DePaola stated that milk products increase the calcium and phosphorus level in plaque thus diminishing demineralization and favoring remineralization.³⁴ Also, the casein present in milk products forms casein phosphopeptide on tryptic proteolysis. These form CPP-ACP complexes which stabilize colloidal calcium and phosphates, maintaining their supersaturation in saliva. These readily diffuse in plaque, favoring remineralization. Intergroup comparison showed consumption of paneer had significantly higher values of calcium and phosphorus than the water group at all times.

The alkaline phosphatase levels in both groups decreased after the intake of candies from baseline. However, our findings contradicted studies by Pandey et al.³⁵ and Gandhi and Damle³⁶ where higher inorganic phosphorus and alkaline phosphatase levels were noted with increased caries whereas study by Vahedi et al.³⁷ showed no relation between dental caries and alkaline phosphatase.

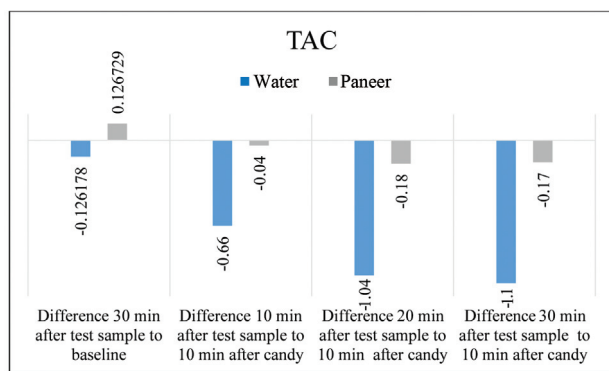
The alkaline phosphatase values increased significantly from 10 to 30 minutes after rinsing with water, and the consumption of paneer with paneer groups showing significantly higher values. Demineralization and remineralization depend on minerals, like calcium and phosphorus, which are influenced by alkaline phosphatase levels. Since our findings contradict most of the studies,^{35,36} further research is needed to evaluate the changes in alkaline phosphatase levels of saliva following the cariogenic challenge and remineralization.

Table 14 Total antioxidant capacity (TAC) level in saliva at baseline, after the consumption of candies and after the consumption of paneer (20 g)

Sr. no.	Paired samples statistics				Paired differences		t	p-Value
	Parameter (TAC)	Mean (mM/L)	n	Standard deviation	Mean (mM/L)	Standard deviation		
1	Baseline	0.887422	30	0.3971692	-0.29355	0.668406	-2.406	0.023 ^b
	10 minutes after candies	1.180975	30	0.5293483				
2	10 minutes after candies	1.180975	30	0.5293483	0.042689	0.59254	0.395	0.696 ^a
	10 minutes after paneer	1.138286	30	0.3472459				
3	10 minutes after candies	1.180975	30	0.5293483	0.181918	0.66374	1.501	0.144 ^a
	20 minutes after paneer	0.999057	30	0.3146654				
4	10 minutes after candies	1.180975	30	0.5293483	0.166824	0.609027	1.5	0.144 ^a
	30 minutes after paneer	1.01415090	30	0.322006250				
5	10 minutes after paneer	1.138286	30	0.3472459	0.13923	0.470108	1.622	0.116 ^a
	20 minutes after paneer	0.999057	30	0.3146654				
6	10 minutes after paneer	1.138286	30	0.3472459	0.124135	0.444355	1.53	0.137 ^a
	30 minutes after paneer	1.01415090	30	0.322006250				
7	20 minutes after paneer	0.999057	30	0.3146654	-0.01509	0.464707	-0.178	0.86 ^a
	30 minutes after paneer	1.01415090	30	0.322006250				
8	Baseline	0.887422	30	0.3971692	-0.12673	0.562472	-1.234	0.227 ^a
	30 minutes after paneer	1.01415090	30	0.322006250				

^ap > 0.05: not significant.^bp < 0.05: significant.**Table 15** Intergroup comparison of changes in salivary total antioxidant capacity (TAC) from baseline after rinsing with water and consumption of paneer (post hoc Tukey's test)

Parameter	I Group	J Group	Mean difference (I-J)	Standard error	p-Value
TAC					
Difference between 30 minute after test sample to baseline	Water	Paneer	-0.25	0.16	0.255943
Difference between 10 minute after test sample to 10 minute after candy	Water	Paneer	-0.61	0.20	0.007934
Difference between 20 minute after test sample to 10 minute after candy	Water	Paneer	-0.85	0.26	0.00384
Difference between 30 minute after test sample to 10 minute after candy	Water	Paneer	-0.93	0.25	0.000944

**Fig. 10** Intergroup comparison of changes in salivary calcium from baseline after rinsing with water and consumption of paneer. TAC, total antioxidant capacity.

The salivary peroxidase system in one part determines the TAC of saliva. It catalyzes the peroxidation of thiocyanate to form hypothiocyanate which inhibits bacterial glycolysis. Many studies by Pandey et al³³ and Mohammed et al³⁸ have shown higher TAC in caries active children. But very few studies have been done on the antioxidants present in dairy products and their effect.

The TAC of saliva acts as a compensatory mechanism against the oxidative stresses produced by bacteria. Our study saw a rise in TAC from baseline after consuming candies, indicating increased bacterial activity.

The values decreased significantly after rinsing with water and consumption of paneer which showed a protective effect. These values were significantly lower than baseline at the end of 30 minutes after rinsing with water.

They were higher than baseline after the consumption of paneer which could be attributed to the inherent antioxidants present in the dairy products. However, the intergroup comparison did not show much difference. Further studies must evaluate the antioxidants present in the dairy product and their role in the oral cavity after consumption.

Conclusion

Thus our study has proved that intake of sweetened candies creates a cariogenic environment conducive for demineralization. The efficacy of dairy products like paneer and consumption of water helps in the reversal of the cariogenic challenge caused by candies as evidenced in our findings.

Conflict of Interest

None declared.

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