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RESEARCH ARTICLE

EFFECT OF NON-FLUORIDATED AND FLUORIDATED MILK ON DENTAL PLAQUE pH CHALLENGED WITH A SWEETENED DRINK AT VARIOUS TIME INTERVALS

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Abstract

Aim: To investigate the effect of non-fluoridated and fluoridated milk on dental plaque challenged with sweetened drink by measuring the plaque pH at various time intervals.

Patients and Methods: A total of 80 children aged 6-8 years who reported to the Department of Pedodontics and Preventive Dentistry, of which 10 children who were interested to participate in the study were included. Children were asked to rinse for 2 min with following solutions: Distilled water, 10% sucrose, non-fluoridated milk, fluoridated milk, 10% sucrose followed by distilled water, 10% sucrose followed by non- fluoridated milk, 10% sucrose followed by fluoridated milk. The supragingival plaque was collected before rinsing and after 5, 10, 15, 20, 25, 30 and 60 min to measure the plaque pH. The values were tabulated and subjected to statistical analysis with one way analysis (ANOVA).

Results: The result of present study shows that the minimum pH drop was almost similar for non-fluoridated and fluoridated milk group at 5 min which is lower than 10% sucrose group but the recovery of pH to baseline value was at faster rate in fluoridated milk group compared to non-fluoridated milk group.

Conclusion: There are parts of world where drinking water cannot be fluoridated due to lack of resources, socio-economic and technical constraints, absence of political will and national policy on oral health. Hence fluoridation of milk can be recommended where fluoride concentration in drinking water is suboptimal and caries experience is significant.

Introduction:-

The microorganisms within dental plaque are highly diverse, and the development of disease is not solely attributable to external species but rather to shifts in the relative proportions of the resident microflora, particularly an increase in mutans streptococci levels.[1] Stephan's classical studies in the early 1940s demonstrated that when dental plaque is exposed to sucrose, acids are rapidly produced. These acids diffuse through the plaque and reach the porous enamel (or exposed dentin), releasing hydrogen ions along the way. This process leads to a swift decline in pH followed by a gradual recovery towards the baseline plaque pH.[2,3]

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The prevention of dental caries can be effectively managed through collaborative efforts involving communities, professionals, and individuals, focusing on reducing sugar consumption and highlighting the beneficial effects of fluorides. Research extensively explores the differential effects of loosely bound versus structurally bound fluoride on preventing caries, emphasizing the crucial role of fluoride bioavailability in the oral environment. Various methods are employed to enhance fluoride's availability for caries prevention, such as tablets, dietary inclusion, or localized application on tooth surfaces.[4]

Milk possesses inherent buffering capabilities against organic acids produced during sugar fermentation. Furthermore, milk proteins adsorbed onto tooth surfaces provide protection against demineralization and also facilitates remineralization processes. Therefore, consuming milk or fluoridated milk following the intake of sugary drinks or foods may aid in reversing the caries process. Studies have shown that the presence of fluoride in milk increases the pH of biofilms, reduces acid formation from lactose fermentation, and influences pH levels in accumulated plaque. However, further investigation is needed to fully understand the immediate effects of milk or fluoridated milk on plaque pH subsequent to exposure to a cariogenic diet.[5]

Previous research has been limited in exploring how combining milk with fluoride can enhance its ability to restore acidic plaque pH to normal levels, thus lowering the risk of dental caries. Therefore, the current study aims to assess how milk and fluoridated milk affect acidic plaque pH following exposure to a sweetened drink at different time intervals.

Patients and Methods:-

A total of 80 children aged 6-8 years presenting to the Department of Pedodontics and Preventive Dentistry were initially screened, of which 10 children who were interested to participate in the study were included. Prior to the study, informed consent was obtained from the parents.

Inclusion criteria:
(1) Good general health, (2) no recent use of medications within the last 15 days and none during the study, (3) no known allergies to milk or fluoride, and (4) no history of orthodontic treatment or wearing orthodontic appliances.

Exclusion criteria:
(1) Children with any systemic disorders, (2) significant gingivitis or periodontitis, and (3) recent use of antibiotics.

Before commencement of the study, a dental examination was conducted to assess the mean decayed, missing, filled surfaces (dmfs) or Decayed, Missing, Filled Surfaces (DMFS). Subsequently, after complete oral prophylaxis, unstimulated saliva was collected to determine saliva flow rate and buffering capacity (Salivary Buffer Kit, GC India Pvt Ltd, Telangana, India). Dental examination and saliva collection was performed by a single trained examiner.

Preparation of 10% sucrose: 10% sucrose solution was prepared by dissolving 10 grams sugar in 100 ml of distilled water such that the final resultant solution by weight contains 10% sugar and 90% water.

Preparation of fluoridated milk:[6] Fluoridated pasteurized milk was prepared by adding a specific quantity of sodium fluoride aqueous solution (Extrapure, Finar Chemicals, Ahmedabad) to milk (Heritage Foods Limited, Vishakapatnam, India). The sodium fluoride stock solution was initially prepared at 1000 ppm by dissolving 2.21 grams of sodium fluoride per 1 liter of distilled water. To achieve a fluoride concentration of 5.0 ppm in the milk, 5 ml of the sodium fluoride aqueous solution was diluted into 1 liter of milk.

The current in-vivo study involved seven test solutions distributed across seven groups (Table 1). All participants were exposed to each test solution group in random sequences, with a 10% sucrose solution used as a sweetened drink. There was a 7-day washout period between each experiment session. Participants were instructed to refrain from oral hygiene for 24 hours and fast overnight before each experiment, conducted in the early morning prior to breakfast.

During each experiment, supra-gingival plaque from the buccal surfaces of the maxillary posterior teeth was randomly collected using a no. 17 spoon excavator to measure baseline plaque pH and resting plaque pH is in the range of 6.3-6.8 as determined by a pH electrode method. Each child rinsed with the experimental solutions for 2

minutes and then expectorated. Supra-gingival plaque specimens were collected randomly at 5, 10, 15, 20, 25, 30, and 60 minutes post-rinsing to assess plaque pH. Collected plaque samples were immediately transferred to 0.4 ml microcentrifuge tubes containing 40 µl of deionized water and centrifuged. To measure pH, the resulting solution was transferred to a beaker with 40 ml of deionized water, and a pH electrode was used to record the pH of all test solutions at 5, 10, 15, 20, 25, 30, and 60 min intervals.

During each experimental session, the average plaque pH of all 10 individuals was plotted against time to generate a pH curve. Three key parameters were derived from the curve: the minimum pH, the maximum pH drop from baseline, and the area under the curve between the plaque pH curve and baseline. Data collected were tabulated and subjected to statistical analysis.

Results:-

The data was subjected to statistical analysis by one way analysis (ANOVA) using SPSS software version 22. A p value of < 0.05 was considered statistically significant.

Table 2, Graph 1 represents the mean plaque pH values measured at various time intervals following exposure to each of the test group solutions. Group 1 exhibited no significant change in plaque pH across all time intervals, maintaining mean pH values consistently around 6.3-6.8, equivalent to the resting pH level. In contrast, Group 2 showed a marked decrease in mean plaque pH, dropping to 5.8 ± 0.23 at the 5-minute interval, approaching critical pH level. Group 3 demonstrated a pH decrease with a mean value of 6.82 ± 0.17 at the 5-minute interval, lower than the negative control (Group 1) and higher than Group 2. Group 4 displayed minimal pH decline with a mean value of 6.80 ± 0.15 at 10 minutes, similar to Group 3. For Group 5, the mean plaque pH was 6.55 ± 0.28 at 5 minutes, lower than Groups 3 and 4. Group 6 showed a plaque pH of 6.5 ± 0.27 at 10 minutes, slightly lower than Group 5. Group 7 exhibited a mean plaque pH of 6.5 ± 0.21 at 5 minutes, similar to Group 6, but showed a faster return to normal pH levels compared to Group 6.

Table 3 represents the average minimum pH drop, maximum pH drop, and area under the curve of plaque pH following exposure to different solutions. The minimum pH drop was similar between non-fluoridated and fluoridated milk groups at 5 minutes, both lower than the 10% sucrose group. However, the fluoridated milk group showed a faster recovery of pH to baseline values over time compared to the non-fluoridated milk group, indicating a reduced rate of demineralization with increasing time until reaching baseline pH values.

Discussion:-

Caries risk is closely associated with increased levels of highly acid-tolerant and acidogenic bacteria in dental plaque, which lower plaque pH. When selecting sites to sample supragingival plaque, it's important to focus on areas most susceptible to caries development. Plaque pH varies between different sites in the mouth due to varying degrees of contact with saliva, which clears substrates and buffers acid.[7]

The resting pH of saliva significantly influences the microbial environment within dental plaque. Within the plaque environment, the resting pH results from a delicate balance between alkali and acid generation. It is generally lowest in interproximal regions, which lack access to saliva once the plaque biofilm thickens to occlude the gingival embrasure beneath the contact points. Fermentation in plaque, particularly in the presence of sucrose, produces significant amounts of lactate, contributing to its cariogenic potential.[3] The high cariogenicity of plaque exposed to sucrose is attributed to the presence of insoluble glucan polymers in its adhesive matrix. Foods high in sucrose often lack nutritional value in terms of fibre, vitamins or minerals, and are termed as “empty calories”.[8]

Saliva volume and flow rate affect its buffering capacity and acid diffusion from plaque. Variations in saliva flow in different areas of mouth contribute to uneven distribution of carious lesions in the mouth. Plaque can generate alkali and neutralize acid, essential for maintaining oral health.[9] Various acids, notably lactic and acetic acids, are rapidly produced in plaque. Plaque pH remains low as long as sugars are available and acid production persists, potentially dropping as low as pH 3.9.[9]

Research strongly supports studying plaque bacteria in biofilm form rather than in aqueous suspensions due to significant differences between the two. Dental caries result from acid-mediated demineralization of tooth enamel, particularly lactic acid produced through microbial fermentation of dietary carbohydrates. Therefore, the reduced

presence of streptococci (especially S. mutans) in biofilms cultivated with fluoridated milk suggests they may have lower cariogenic potential compared to biofilms grown in regular milk. Plaque with reduced cariogenic potential typically utilizes lactate as a carbon and energy source, converting it into propionic acid, which is less acidic.Although the specific location of mutans streptococci within plaque and their role in caries development have not been extensively studied, it is likely that their demineralizing impact is greater when they are closer to the enamel surface.[1]

In 1953, Swiss pediatrician Ziegler recommended using milk as a vehicle for fluoride (F) delivery, and subsequent studies have confirmed the caries-preventive benefits of fluoride in milk.[10] Milk is a complex colloidal mixture containing proteins, fats, lactose, minerals, and various other constituents, both in suspension and solution.[11]In school-based milk fluoridation programs, children typically consume 200 ml of milk at a concentration of 5.0 mg F, providing a daily fluoride dose of 1.0 mg F per school day. This concentration is comparable to fluoride levels used in community water fluoridation (0.7–1.2 parts per million). For younger children in nursery settings, the fluoride dose is adjusted accordingly, generally ranging from 0.25 to 0.5 mg per day.Fluoridated milk at a concentration of 2.5 ppm can effectively prevent demineralization of dental enamel.[6]

The understanding of fluoride's role in preventing dental caries has evolved, shifting towards recognizing the importance of its topical effects despite systemic benefits from systemic fluoride agents. Fluoridated milk consumption helps inhibit enamel demineralization and promote remineralization. Within 30-60 minutes of consuming fluoridated milk, levels of fluoride in both whole saliva and dental plaque increase due to its presence in the mouth and absorption into salivary secretions. This dual action of fluoride in milk- both systemic and topical is similar to fluoride in water.[12] Milk serves as an effective vehicle for delivering fluoride to children, and the process of adding fluoride to milk is straightforward without altering its taste or other characteristics. The slow fermentable lactose in milk is less cariogenic than sucrose, and the proteins and fats in milk may also have a cariostatic effect.[13]

In the present study, maximum pH drop was observed in the 10% sucrose group, with a mean plaque pH of 5.8. This finding is consistent with research by Mor BM et al., who observed pH changes in three-day-old plaque after rinsing with various solutions including 5% sucrose, 5% lactose, milk, and milk with 4 ppm fluoride. Milk was found to be the least acidogenic, as only one subject reached a minimum pH after six minutes compared to several subjects within three minutes with sucrose rinses. Milk often produced a initial rise in pH, with sucrose pH began to fall immediately.[14]

The current study also corroborates with findings from research conducted by Moynihan PJ et al., who examined whether glucose polymers, administered as 10% solutions in water, cow's milk, or a milk substitute (Calogen), have acidogenic effects and if these solutions can lower plaque pH to levels associated with enamel demineralization. When comparing sucrose and glucose polymer solutions in milk, sucrose consistently induced a more pronounced drop in pH and showed a tendency towards a smaller pH area. However, differences in the minimum pH reached and the duration below pH 6 were not statistically significant. pH levels of 5.5 or below were observed in five subjects following rinses with both the sucrose/milk and glucose polymer/milk solutions.[15]

In the present study, a reduction in plaque pH was observed in groups rinsed with both fluoridated and non-fluoridated milk, with mean plaque pH values of 6.8 and 6.5 respectively, which were similar to baseline values. However, the recovery to resting pH was faster in the fluoridated milk group. These findings are consistent with those of Stosser L et al., who investigated the caries-preventive effects of non-fluoridated and fluoridated milk, and compared different fluoride compounds added to milk. Their study on rats showed that those receiving non-fluoridated milk or distilled water had a significantly higher incidence of dental caries compared to those receiving fluoridated milk.[16] Similarly, the present study aligns with research by Malinowski M et al., who examined the dose-response relationship of fluoride in milk on enamel demineralization and remineralization using transverse microradiography (TMR). They found that control groups with 0 ppm fluoride demonstrated net demineralization, whereas all fluoridated groups showed remineralization, with greater remineralization observed with higher fluoride concentrations in milk.[10]

The ability of fluoridated whole milk to inhibit lesion progression underscores the concept that fluoride functions more as a treatment agent for halting and remineralizing caries lesions rather than solely as a preventive measure in a causal protocol. The mechanisms behind the efficacy of fluoridated milk may involve three main factors: fluoride binding to calcium, forming a potent reservoir of loosely bound fluoride; milk's inherent contribution to

remineralization due to its high calcium and phosphorus content; and milk proteins, such as protease-peptone fractions, adsorbing onto tooth surfaces to inhibit rapid demineralization below critical pH levels. From an experimental perspective, these findings suggest that milk serves as an effective nutritional carrier of fluoride while concurrently offering protective properties against acid attacks through minor milk proteins and peptides.[17]

Tables And Graph

Table 1:- Test solution groups.

Groups	Test solution groups and procedures
1	Negative control- 2 min with10 ml distilled water
2	Positive control – 2 min with 10 ml of 10% sucrose
3	Positive control – 2 min with 10 ml of whole milk
4	Positive control – 2 min with10 ml of fluoridated whole milk
5	Positive control – 2 min with 10 ml of 10% sucrose, spit it out, then 2 min with distilled water
6	Experiment - 2 min with 10 ml of 10% sucrose, spit it out, then 2 min with 10 ml of whole milk

Table 2:- Mean pH values at various time intervals after a challenge with all test group solutions.

Time	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Baseline	7.17±0.15	7.30±7.0.18	7.23±0.15	7.28±0.15	7.01±0.15	7.30±0.16	7.16±0.15
5 min	7.30±0.27	**5.80±0.23**	**6.82±0.17**	6.90±0.17	**6.55±0.28**	6.78±0.27	**6.50±0.21**
10 min	7.25±0.21	6.10±0.23	6.97±0.11	**6.80±0.15**	6.62±0.33	**6.50±0.27**	6.71±0.18
15 min	7.18±0.29	6.54±0.20	7.15±0.22	7.21±0.21	6.71±0.34	6.58±0.20	6.73±0.21
20 min	7.20±0.45	6.61±0.23	7.30±0.39	7.28±0.11	6.82±0.29	6.63±0.33	6.65±0.28
25 min	7.28±0.20	6.70±0.24	7.23±0.27	7.18±0.33	6.70±0.29	6.72±0.20	6.72±0.27
30 min	**7.15±0.24**	6.88±0.35	7.18±0.18	7.16±0.30	7.00±0.27	7.18±0.21	7.18±0.17
60 min	7.20±0.15	7.10±0.25	7.50±0.08	7.31±0.23	7.03±0.21	7.08±0.10	7.15±0.17
P value	0.001 S	<0.001 S	<0.001 S	<0.001 S	<0.001 S	<0.001 S	<0.001 S

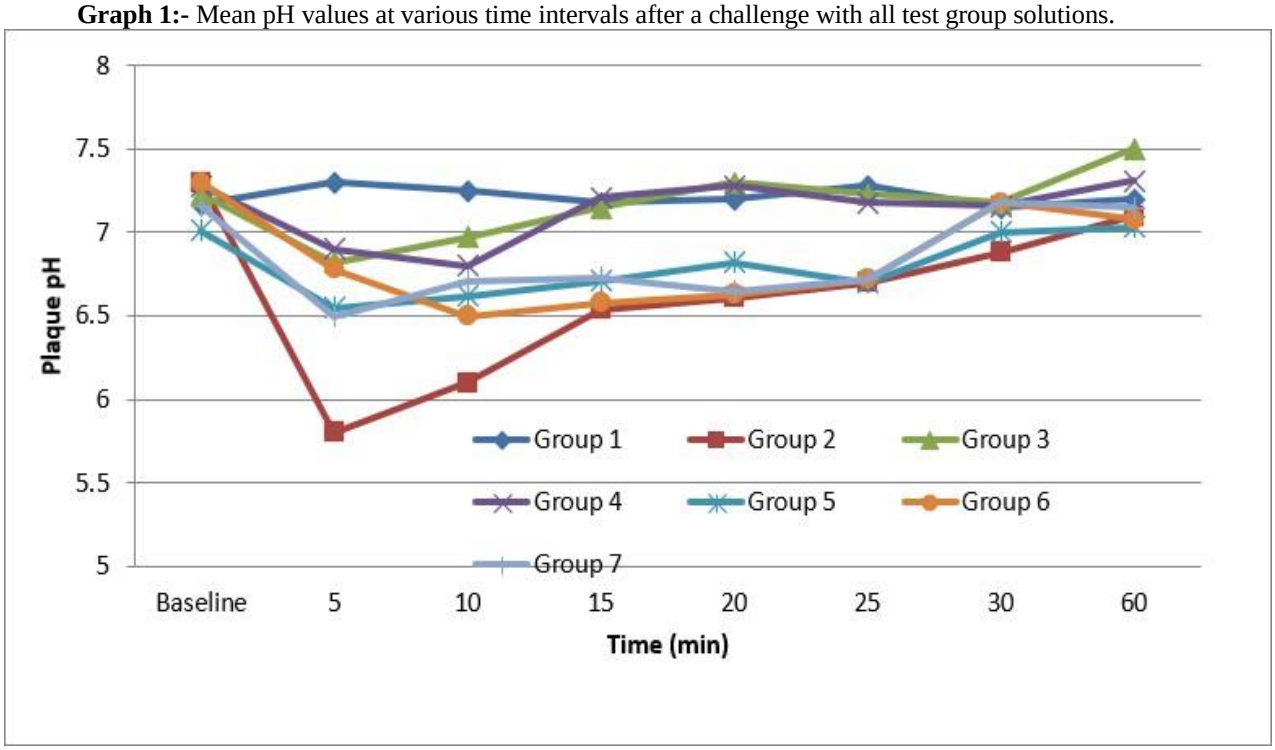
SD=Standard deviation; pvalue=Probability value

Table 3:- Average minimum pH drop, maximum pH drop and area under curve of plaque pH after a challenge with different solutions.

Time	Minimum pH drop	Maximum pH drop	Area under curve
	Mean±SD	Mean±SD	Mean±SD
Group1	7.15±0.24	0.15±0.03	-0.81±0.53
Group2	5.80±0.23	1.50±0.05	4.62±0.14
Group3	6.82±0.17	0.68±0.09	0.28±0.55
Group4	6.80±0.15	0.51±0.08	0.72±0.24
Group5	6.55±0.28	0.48±0.07	1.38±0.29
Group6	6.50±0.27	0.80±0.11	3.12±0.47
Group7	6.50±0.21	0.68±0.04	1.55±0.40

pH= Standard for potential of hydrogen; SD=Standard deviation

Graph 1:- Mean pH values at various time intervals after a challenge with all test group solutions.



Conclusion:-

The present study demonstrated lowest pH value of dental plaque was seen after rinsing with 10% sucrose and recovery of acidic plaque pH after rinsing with fluoridated milk was faster compared to non fluoridated milk.

Adjustments in lifestyle that reduce the production of highly destructive acids by plaque, while allowing for the generation of weaker acids that can be buffered more effectively, are crucial for maintaining a healthy equilibrium between the plaque biofilm and the tooth surface.

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