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TO EVALUATE THE CARIOGENIC POTENTIAL OF NATURAL AND ARTIFICIAL MILK BEVERAGES BY STREPTOCOCCUS MUTANS BIOFILM MODEL IN PRIMARY TEETH : AN IN-VITRO STUDY

Divya madhuri¹, Sridhar. M², Naseemoon Shaik³, Sravanthi J⁴, Pravalika B⁵, Himasree K⁶.

^{1,5,6}year post graduate student, Department of Pedodontics & Preventive Dentistry MNR Dental College, Sangareddy, Telangana.

²Professor & Head of the Department, Department of Pedodontics & Preventive Dentistry MNR Dental College, Sangareddy, Telangana.

^{3,4}Associate Professor, Department of Pedodontics & Preventive Dentistry MNR Dental College, Sangareddy, Telangana.

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ABSTRACT

Background: Dental caries remains a prevalent chronic disease in children, influenced by diet, microbial activity, and oral hygiene. Milk is a widely consumed beverage with both protective and cariogenic potential, depending on its composition and sugar content. This study evaluated the cariogenic effects of natural and flavoured milk beverages on primary teeth using an *in vitro* *Streptococcus mutans* biofilm model.

Materials and Methods: Sixty caries-free primary teeth were sectioned into enamel slabs and randomly allocated into six groups: cow, buffalo, goat, strawberry-flavoured, vanilla-flavoured milk, and saline control (n=10 each). Enamel slabs were inoculated with *S. mutans* and exposed to the respective beverages three times daily for three days. Baseline and post-exposure surface microhardness were measured using the Brinell hardness test. Percentage surface hardness loss was calculated, and data were analyzed using one-way ANOVA with Tukey's post hoc test and paired t-tests (p<0.05).

Results: Baseline microhardness was comparable across all groups (p>0.05). Natural milk types (cow, buffalo, goat) caused moderate enamel demineralization, whereas flavoured milk beverages led to significantly higher microhardness loss (p<0.05). Tukey's post hoc analysis confirmed that sweetened milk groups exhibited greater cariogenic potential compared to natural milk and the control.

Conclusion: Natural milk poses a lower risk for enamel demineralization due to its buffering capacity and absence of added sugars. Sweetened milk beverages significantly increase cariogenic risk, particularly with frequent consumption. Limiting sugary milk drinks and promoting proper oral hygiene are essential strategies to reduce caries risk in children.

Introduction :

Dental caries is the most common non-communicable disease globally and remains a major public health challenge despite preventive strategies. The World Health Organization

(WHO) Global Oral Health Status Report (2022) estimates that 3.5 billion people suffer from oral diseases, including 2 billion with caries in permanent teeth and 514 million children with caries in primary teeth[1]. The burden is disproportionately higher in middle-income countries.

Corresponding author: Dr. Naseemoon Shaik

Email id: dr.naseemoonshaik@gmail.com

In India, caries prevalence is alarming, with nearly 62% of adults above 18 years and 52% of children between 3 and 18 years affected [2].

Historically, the dietary shift during the Neolithic era from unprocessed fibrous foods to cereal-based diets increased caries prevalence, as confirmed by archaeological findings from Central Germany [3]. Caries is a multifactorial disease involving the interaction of dental biofilm, dietary sugars, host factors, and time [4]. Bacteria such as *Streptococcus mutans* metabolize fermentable carbohydrates into acids, lowering plaque pH and initiating enamel and dentin demineralization. Without adequate remineralization, cavitation occurs. Saliva plays a protective role, but frequent sugar intake, poor oral hygiene, and inadequate fluoride shift the balance toward disease progression [5].

Children and adolescents are at higher risk due to thinner enamel, irregular eating habits, and preference for sugary foods and drinks [6]. The increasing popularity of alternative milk beverages in children's diets—many of which contain added sugars—raises concerns about their cariogenic potential. Although the cariogenic effects of sugary foods and beverages are well-documented, limited evidence exists regarding the impact of natural versus artificial milk beverages on caries development. With growing consumption of plant-based and flavored milk alternatives in children, it is crucial to evaluate their role in biofilm formation, acidogenic potential, and pH changes to understand their contribution to dental caries. So aim of the present study was to evaluate the cariogenic potential of natural and artificial milk beverages using a *Streptococcus mutans* biofilm model in primary teeth.

Methodology:

This comparative in vitro study was approved by the Institutional Ethics Committee, MNR Dental College and Hospital (MNRDCH/IEC/2022-23/09). The study evaluated the cariogenic potential of five different milk types on primary tooth enamel.

Sample Size Determination:

Sample size was calculated using G*Power (v3.1.9.7) for one-way ANOVA with fixed effects. With an effect size of 0.4953, $\alpha = 0.05$, power = 0.80, and six groups, 60 enamel samples were required (10 per group).

Specimen Selection:

Sixty extracted, caries-free primary teeth with intact enamel were included. Exclusion criteria were: teeth with pathology or gross decay (>80% crown loss). Extracted teeth were stored in sterile isotonic saline until use [7].

Preparation of Enamel Slabs:

Only the crown portions were used. Slabs (2–3 mm thickness) were sectioned using a diamond disc under continuous water cooling to avoid thermal damage [8]. Each slab was mounted in cold-cure acrylic resin with enamel surfaces exposed and color-marked for group identification.

Randomization and Grouping

Specimens were randomly assigned to six groups (n=10 each):

Group 1 Control Saline (n=10);

Test Group 2 Cow milk (n=10)

Test Group 3 Goat milk (n=10)

Test Group 4 Buffalo milk (n=10)

Test Group 5 Strawberry milk (n=10)

Test Group 6 Vanilla milk (n=10)

Baseline Microhardness Measurement

Enamel hardness was assessed using a Brinell hardness tester (load 60–125 g, dwell 30–60 s) [9].

Bacterial Inoculation and Beverage Exposure

Slabs were inoculated with *Streptococcus mutans* (ATCC 25175) in BHI broth with 1% sucrose ($\sim 1 \times 10^8$ CFU/mL) and incubated at 37°C for 24 h to allow biofilm formation [10]. Specimens were then exposed three times daily for three consecutive days to their assigned milk beverages for 30 min per cycle, rinsed with sterile saline, and coated with artificial saliva [11]. Post-exposure, slabs were disinfected with 97% ethanol and sterilized in an ultrasonicator with double-deionized water.

Post-Exposure Microhardness Measurement

Percentage surface hardness loss was calculated as:
 $\% \text{Loss} = \frac{\text{Initial} - \text{Final Hardness}}{\text{Initial Hardness}} \times 100$

Results :

Data were analyzed using **IBM SPSS version 22**. Intergroup comparisons were performed using **one-way ANOVA** followed by **Tukey's post hoc test**, and within-group comparisons between initial and final surface microhardness were performed using **paired t-tests** ($p < 0.05$ considered significant). Initial surface microhardness (ISM) was comparable across all groups ($p = 0.706$), indicating uniform baseline enamel hardness. Post-exposure microhardness (FSM) also showed no significant intergroup differences ($p = 0.934$). Within-group analysis revealed statistically significant reductions

in FSM for Test Groups 4 and 5 (p=0.007 and 0.04, respectively), suggesting these milk types had a measurable impact on enamel hardness. Other groups did not show significant within-group changes (Table 1).

Table 1: Initial and Final Surface Microhardness (Mean ± SD) and Within-Group Comparison

Groups	ISM (Mean ± SD)	FSM (Mean ± SD)	p-value (paired t-test)
Control	1.00 ± 0.61	0.94 ± 0.65	0.06
Test Group 1	0.83 ± 0.36	0.90 ± 0.42	0.227
Test Group 2	1.23 ± 0.57	1.06 ± 0.46	0.323
Test Group 3	1.10 ± 0.57	1.14 ± 0.57	0.526
Test Group 4	1.11 ± 0.66	0.92 ± 0.66	0.007*
Test Group 5	1.17 ± 0.34	0.96 ± 0.64	0.04*

*ISM – Initial Surface Microhardness; FSM – Final Surface Microhardness; p<0.05 significant

The mean percentage loss of enamel hardness differed significantly between groups (p=0.04). Test Group 3 (Buffalo milk) and Test Group 1 (Cow milk) experienced the highest microhardness loss (-105.3 and -104.5), whereas Test Groups 4 and 5 (Strawberry and Vanilla milk) showed the lowest loss (-76.0 and -78.8). This indicates that different milk types exert varied effects on enamel demineralization (Table 2).

Table 2: Loss of Surface Microhardness (Mean ± SD) – Intergroup Comparison

Groups	Mean Loss ± SD	p-value (ANOVA)
Control	-89.2 ± 16.1	0.04*
Test Group 1	-104.5 ± 28.1	
Test Group 2	-98.1 ± 36.7	
Test Group 3	-105.3 ± 25.6	
Test Group 4	-76.0 ± 20.63	
Test Group 5	-78.8 ± 20.54	

*One-way ANOVA; p<0.05 significant

Pairwise comparisons identified significant differences between Test Group 1 vs Test Groups 4 and 5 (p=0.016 and 0.029) and Test Group 3 vs Test Groups 4 and 5 (p=0.013 and 0.024), confirming that treatments in Groups 1 and 3 were more cariogenic compared to Groups 4 and 5. No significant

differences were observed among the remaining pairs (Table 3).

Table 3: Tukey’s Post Hoc Pairwise Comparisons for Surface Microhardness Loss

Comparison	Mean Difference	p-value
Control vs Test 1	15.27	0.187
Control vs Test 2	8.93	0.438
Control vs Test 3	16.08	0.165
Control vs Test 4	-13.21	0.253
Control vs Test 5	-10.46	0.364
Test 1 vs Test 4	-28.48	0.016*
Test 1 vs Test 5	-25.73	0.029*
Test 3 vs Test 4	-29.29	0.013*
Test 3 vs Test 5	-26.54	0.024*

*p<0.05 significant

Discussion:

Dental caries is a multifactorial disease influenced by diet, oral hygiene, and microbial activity. In this study, the cariogenic potential of commonly consumed milk beverages—including cow, buffalo, goat, and flavoured milk (strawberry and vanilla)—was evaluated using primary teeth enamel slabs and *Streptococcus mutans* biofilm models. The findings highlight that the type of milk and the presence of added sugars significantly affect enamel demineralization. Natural milk types such as cow, buffalo, and goat milk demonstrated moderate effects on enamel microhardness. This aligns with previous research indicating that bovine milk exhibits low cariogenicity due to its high calcium, phosphate, and casein content, which promote remineralization and buffer acidic challenges (Pradhan et al., 2024)[12]. Goat milk, with its smaller fat globules and bioactive peptides, showed comparable protective effects, consistent with Haenlein [13], who reported that goat milk supports enamel integrity while providing additional nutritional benefits. Similarly, Thimma Reddy et al. [14] observed minimal cariogenic effects from natural milk types in children, emphasizing the relatively safe profile of milk when consumed without added sugars. In contrast, flavoured milk beverages containing added sugars exhibited the highest enamel microhardness loss. Test Groups 4 and 5 (strawberry and vanilla milk) demonstrated significant reductions in surface hardness compared to natural milk. These findings are in agreement with Huang [15] and Wimolsantirungreung [16], who reported that sweetened milk and plant-based beverages enhance *S. mutans* biofilm formation, increase acid production, and accelerate enamel demineralization. The current study confirms that the sugar content, rather than milk type alone, is the primary driver of

cariogenicity in children's beverages.

The study also highlights the role of frequency and duration of exposure. The enamel slabs were subjected to simulated daily dietary patterns, reflecting real-life consumption. This approach supports findings by Signori et al. [17] reported that sucrose addition to milk significantly increased enamel hardness loss compared to unsweetened milk. These studies underscore the synergistic effect of sugar content and frequency of exposure on enamel demineralization.

The protective properties of natural milk are further supported by the role of casein and calcium-phosphate complexes, which can inhibit demineralization and promote remineralization [18]. The present findings confirm that natural milk types have a moderate cariogenic potential, whereas flavoured and sugar-enriched beverages are significantly more aggressive in demineralizing enamel. This aligns with Srivoha et al., who reported that sweetened milk substitutes accelerated *S. mutans* biofilm formation and enamel demineralization, while unsweetened milk induced flatter biofilms with limited surface loss [19].

Using primary teeth enamel in this study enhances clinical relevance, as these teeth are more susceptible to caries due to thinner enamel and reduced saliva buffering capacity. This approach provides a realistic assessment of the structural impact of milk beverages on children's teeth, complementing previous studies that primarily employed microtiter biofilm assays without direct evaluation of enamel [20]. Overall, the results of this study reinforce that while natural milk remains relatively safe for children's dental health, the consumption of flavoured milk beverages, particularly those high in added sugars, poses a substantial risk for enamel demineralization and caries development. Caregivers and dental professionals should consider both the sugar content and frequency of intake when recommending milk beverages for children.

Limitations of the study include the use of a single-species biofilm, lack of dynamic saliva flow, pellicle formation, and mechanical cleaning, which may affect the extrapolation of results to real-world oral conditions. Despite these limitations, the study provides valuable insight into the relative cariogenicity of commonly consumed milk beverages and underscores the importance of sugar content in modulating enamel demineralization.

Conclusion:

The cariogenic potential of milk beverages is largely determined by sugar content and their capacity to support *Streptococcus mutans* biofilm formation. Natural milk, with its buffering properties and lack of added sugars, presents a lower risk for enamel demineralization. In contrast, sweetened milk beverages significantly increase the likelihood of caries, especially with frequent consumption or inadequate oral hygiene. Caregivers should choose milk wisely, and dental

professionals should reinforce limiting sugary drinks and maintaining proper oral hygiene to protect children's dental health.

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