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Effect of xylitol, propolis and probiotics on dental plaque

Ralitsa Bogovska-Gigova

Abstract:
Objective*: This study evaluated and compared the effects of xylitol lollipops, propolis mouthwash, and probiotic tablets on plaque microbial counts of* Mutans Streptococci *and* Lactobacilli *in children aged 7–12 years to assess their potential as preventive measures against dental caries.*
Methods*: Forty children were enrolled in a four-stage study. Stage 1 (control) involved standard oral hygiene without prophylactic products. In Stages 2, 3, and 4, children used probiotic tablets, xylitol lollipops, or propolis mouthwash, respectively, once daily for 14 days, with one-month washout periods between stages. Plaque samples from maxillary incisors and canines were collected, diluted, and inoculated onto Rogosa agar (*Lactobacilli*) and mitis-salivarius-bacitracin agar (*Mutans Streptococci*). Colony-forming units (CFUs) were quantified after 72 hours of incubation at 37°C. Microbial counts were assessed at the beginning and end of each stage.*
Results*: Baseline microbial counts showed 25.70–28.62 CFU/mL for* Mutans Streptococci *and 12.62–13.87 CFU/mL for* Lactobacilli*, with no significant changes during the control stage. Xylitol lollipops and propolis significantly reduced both* Mutans Streptococci *and* Lactobacilli *14 days. Probiotic tablets significantly reduced* Mutans Streptococci*, but not* Lactobacilli*.*
Conclusion*: Xylitol lollipops were the most effective in reducing cariogenic microorganisms, followed by propolis mouthwash, while probiotic tablets selectively reduced* Mutans Streptococci*. These products show promise as adjunctive preventive strategies for dental caries in high-risk children.*
Key words: *microorganisms, xylitol, propolis, probiotics*

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I. Introduction:

Dental caries remains a major health concern in both developed and developing countries, affecting approximately 60–90% of school-aged children and adults [1]. It is the most prevalent oral disease in childhood, with dental biofilm identified as a primary risk factor for its development [2]. The etiology of caries involves a disruption of oral microbiome homeostasis, creating conditions favorable for disease progression [3]. Mutans Streptococci (MS) play a critical role in caries development due to their physiological traits, including the ability to colonize tooth surfaces, synthesize insoluble polysaccharides from sucrose, rapidly produce acids from various sugars (acidogenic), and thrive in acidic environments (aciduric). Carious lesions primarily result from lactic acid production, though other acids are also generated as co-products [4]. This acidity leads to enamel demineralization and disrupts biofilm homeostasis, promoting the growth of acidogenic and aciduric species, such as Lactobacilli [5]. By synthesizing large amounts of extracellular polysaccharides from sucrose, MS enhance plaque formation, limiting saliva's access to the plaque and hindering acid diffusion, thus increasing the biofilm's pathogenic potential. Advances in molecular biology have shown that caries initiation and progression involve a broader consortium of microorganisms, including Actinomyces, Abiotrophia, Atopobium, Bifidobacterium, Lactobacillus, and Veillonella, acting synergistically [5, 6].

Despite preventive efforts, the global prevalence of dental caries remains high, necessitating expanded strategies for its prevention and control. Incorporating probiotics, xylitol, and propolis into food products offers a promising approach to reduce pathogenic microorganisms in the oral cavity, opening new avenues for caries prevention and treatment [7].

Probiotics promote oral health by maintaining microbial balance [8]. They produce metabolites like biosurfactants, bacteriocins, and extracellular polysaccharides, inhibit adhesion and colonization, and downregulate virulence genes associated with biofilm formation by cariogenic pathogens [9].

Propolis, a natural resin collected by honey bees, varies in composition based on climate, season, location, and bee species [10]. Its well-documented anti-inflammatory, antioxidant, antiulcer, antitumor, antidiabetic, cardioprotective, and local anesthetic properties make it valuable in medical applications [11]. Propolis also exhibits bacteriostatic, bactericidal, and anti-adhesive effects against various microorganisms, reducing dental biofilm formation [12, 13, 14].

Xylitol, a five-carbon sugar alcohol found in fruits and plants, has a crystalline structure [15]. Sugar alcohols are non-acidogenic or hypo-acidogenic, making them minimally or non-cariogenic [16]. Xylitol inhibits

the growth of oral streptococci [17]. Mutans Streptococci cannot use xylitol for energy production; instead, they metabolize it into xylitol-5-phosphate, which disrupts glucose metabolism to lactate (the primary plaque acid) by inhibiting glycolytic enzymes, leading to reduced acid production and an energy-wasting cycle for MS [17].

Aim: The study aimed to evaluate and compare the effects of oral probiotic tablets, propolis mouthwash, and xylitol lollipops on the microbial count of intraoral cariogenic microorganisms (Lactobacilli and Mutans Streptococci) in children aged 7–12 years and to assess their potential as preventive measures against dental caries.

II. Materials and Methods

The study included 40 children aged 7–12 years with a medium to high risk of developing caries. Inclusion criteria were as follows: children free of systemic diseases, willing to follow hygiene and nutritional recommendations during the study, not allergic to milk or dairy products, and not taking antibiotics or corticosteroids during the study period.

The study was conducted in four stages, each assessing the effect of different products:

- **Stage 1 (Control):** Children followed standard oral hygiene and dietary practices without additional prophylactic products, establishing a baseline for subsequent stages.
- **Stage 2:** Children took oral probiotic tablets once daily for 14 days.
- **Stage 3:** Children consumed xylitol lollipops once daily for 14 days.
- **Stage 4:** Children used propolis mouthwash once daily for 14 days.

A one-month washout period separated each stage, during which children refrained from using prophylactic products and adhered to their usual oral hygiene and dietary routines to prevent carryover effects from previous products. For stages 2, 3, and 4, each child received the respective product with instructions for once-daily use over 14 days. Control examinations to assess microbial counts were conducted in the morning between 8:00 and 11:00 a.m. Participants were instructed to avoid food and drinks (except water) for at least one hour before the visit and to refrain from brushing their teeth on the morning of the examination.

Methodology for Quantifying Cariogenic Microorganisms in Dental Plaque

To quantify cariogenic microorganisms (Lactobacilli and Mutans Streptococci) in dental plaque, plaque samples were collected from the maxillary incisors and canines (temporary or permanent) using a scraping technique. Each sample was immediately placed in 1 mL of sterile saline in an Eppendorf tube to prevent desiccation. Sample dispersion was achieved by vortexing the tubes for 2 minutes at 3000 RPM using a Biosan V-1 Plus vortex mixer.

Prior to inoculation, serial tenfold dilutions (from 1:10 to $1:10^5$) were prepared to enable accurate single-colony counting. The diluted samples were inoculated onto selective nutrient agar media specific for cariogenic microorganisms. Rogosa agar was used for Lactobacilli cultivation, while mitis-salivarius-bacitracin (MSB) agar was used for Mutans Streptococci. Cultures were incubated at 37°C for 72 hours in an anaerobic atmosphere consisting of 95% hydrogen and 5% carbon dioxide.

After incubation, colony-forming units (CFUs) were quantified by manually counting colonies based on their characteristic morphology: small, round, blue colonies on the respective agars. The microbial load was determined by calculating the number of CFUs per sample.

Quantification of cariogenic microorganisms was performed at multiple time points:

- At the beginning and end of Stage 1 (control stage, no prophylactic products).
- At the beginning and end of Stage 2 (probiotic tablet intervention).
- At the beginning and end of Stage 3 (xylitol lollipop intervention).
- At the beginning and end of Stage 4 (propolis mouthwash intervention).

III. Results:

Table 1 shows the average CFU values among the children studied at the beginning of the study – on the first and 14th day.

Table 1. *Amount of mutans streptococci and lactobacilli – baseline*

CFU / MO	1 day (1) Mean ± SD	14 day (2) Mean ± SD	Paired Samples test
Mutans streptococci	28.62 ± 14.22	25.70 ± 11.53	T=1.043, p=0.303
Lactobacillus	12.62 ± 9.43	13.87 ±7.22	T=0.184, p=0.115

At the initial stage (control) of the study, the microbial counts of cariogenic microorganisms ranged from 25.70 to 28.62 CFU/mL for Mutans Streptococci and from 12.62 to 13.87 CFU/mL for Lactobacilli. These data indicate high and comparable levels of cariogenic microorganisms at baseline. After 14 days of standard oral hygiene and dietary practices (control stage), changes in the counts of Mutans Streptococci and Lactobacilli were minimal and lacked statistically significant differences.

Table 2 presents the changes in microbial counts after 14 days of daily probiotic tablet use.

Table 2. *Effect of Probiotic Tablets on Microbial Counts*

CFU / MO	1 day Mean ± SD	14 day Mean ± SD
Mutans streptococci		
Baseline	28.62 ± 14.22	25.70 ± 11.53
Probiotics	31.75 ± 18.51	16.32 ± 9.58
Paired Samples test	T=1.127, p=0.145	T=4.335,p=0.000
Lactobacillus		
Baseline	12.62 ± 9.43	13.87 ± 7.22
Probiotics	11.56 ± 11.15	13.21 ± 6.91
Paired Samples test	T=2.427, p=0.346	T=2.348,p=0.671

Probiotic tablets have the potential to reduce the levels of mutans streptococci significantly with regular use. The levels of lactobacilli remained unchanged, with no significant differences between the groups.

Table 3 presents the change in microbial counts after 14 days of consuming xylitol lollipops.

Table 3. *Effect of xylitol lollipops on the number of microorganisms*

CFU / MO	1 day Mean ± SD	14 day Mean ± SD
Mutans streptococci		
Baseline	28.62 ± 14.22	25.70 ± 11.53
Xylitol lollipop	27.25 ± 12.21	12.97 ± 7.23
Paired Samples test	T=1.467, p=0.151	T=5.442,p=0.000
Lactobacillus		
Baseline	12.62 ± 9.43	13.87 ± 7.22
Xylitol lollipop	10.99 ± 10.12	7.31 ± 5.61
Paired Samples test	T=4.127, p=0.566	T=3.128,p=0.000

Daily consumption of xylitol lollipops for 14 days significantly reduced the microbial counts of cariogenic microorganisms in dental plaque. These reductions were supported by statistically significant differences.

Table 4 presents the changes in microbial counts after 14 days of daily gargling with a propolis solution.

Table 4. *Effect of Propolis Solution on Microbial Counts*

CFU / MO	1 day Mean ± SD	14 day Mean ± SD
Mutans streptococci		
Baseline	28.62 ± 14.22	25.70 ± 11.53
Propolis	27.72 ± 15.49	18.91 ± 12.62
Paired Samples test	T=0.690, p=0.494	T=3.091,p=0.004
Lactobacillus		
Baseline	12.62 ± 9.43	13.87 ± 7.22
Propolis	11.77 ± 4.56	8.41 ± 6.44
Paired Samples test	T=2.333, p=0.789	T=0.668,p=0.000

Propolis gargle solution reduces the amount of cariogenic microorganisms in plaque after 14 days of administration, although to a lesser extent than xylitol lollipops. Statistically significant differences support the data.

IV. Discussion:

Our study evaluates and compares the in vivo effects of widely used over-the-counter products (xylitol lollipops, propolis mouthwash, and probiotic lozenges) on plaque microbial counts of Lactobacilli and Mutans Streptococci.

Effect of Probiotic Tablets

A study conducted two years ago reported that, among three groups using probiotics, chlorhexidine, or an herbal gargle solution, there was a significant reduction in salivary Mutans Streptococci and an increase in salivary pH over 14 days [18]. Salivary levels of S. mutans are directly correlated with the number of colonized dental sites and their proportion in dental plaque. A positive correlation exists between salivary Mutans Streptococci concentrations and dental caries incidence. Pannu et al. found that individuals with lower salivary Mutans Streptococci levels had significantly fewer carious surfaces [19]. A study in India involving daily probiotic supplementation for one year showed an 87% reduction in Streptococci and a 67% increase in Lactobacillus in saliva [20]. Similar findings were reported by Ahola et al. after three weeks of probiotic supplementation via cheese [21]. An in vitro study demonstrated that yogurt containing live Lactobacillus bacteria selectively reduced Mutans Streptococci, suggesting a bactericidal effect on S. mutans in vivo [22]. Other studies have also reported reduced S. mutans levels in the oral cavity following probiotic liquid or tablet use [23], consistent with our findings (Table 2). Arezoo et al. found that surfactants from probiotic bacteria (Lactobacillus acidophilus, Lactobacillus fermentum, and Lactobacillus rhamnosus, present in the probiotic tablet studied) reduce S. mutans adhesion to surfaces [24]. Probiotic therapy appears effective for maintaining oral health and protecting against caries by significantly reducing cariogenic pathogen levels [20]. Our data confirm that regular use of probiotic tablets significantly reduces Mutans Streptococci levels.

Effect of Xylitol Lollipops

Some authors suggest that xylitol acts as an oral prebiotic, reducing Mutans Streptococci levels and enhancing microbial resistance to dysbiosis [25]. Others note that plaque in individuals consuming xylitol is less adhesive due to reduced Streptococci and lower extracellular polysaccharide content [26]. Chavan et al. investigated the effects of chewing gum containing xylitol on salivary S. mutans levels [27]. They concluded that 100% xylitol-sweetened chewing gum, chewed four times daily for 10 minutes over 21 days, significantly reduced salivary S. mutans, potentially aiding caries control in at-risk patients [27]. Another three-week study on xylitol chewing gum found reduced salivary Mutans Streptococci but no significant effect on Lactobacilli [28]. Our data indicate that xylitol lollipops significantly reduced cariogenic microorganisms in plaque after 14 days of daily use (Table 3). Plaque bacteria cannot ferment xylitol, unlike sugars such as sucrose or glucose. Xylitol also increases ammonia and amino acid concentrations while reducing lactic acid production in plaque, neutralizing plaque acids and fostering a less cariogenic oral environment [29]. Additionally, xylitol may stimulate salivary enzyme production, inhibiting bacterial growth in dental plaque [30].

Effect of Propolis Mouth Rinse

Propolis mouthwash may reduce dental plaque formation by inhibiting microbial glucosyltransferase activity, particularly through propolis SNB-RS [31]. Glucosyltransferases are critical enzymes for plaque accumulation, as water-insoluble glucans facilitate bacterial adhesion to teeth and strengthen bonds between Streptococci and the dental pellicle, increasing plaque buildup. Apigenin, a 4',5,7-trihydroxyflavone in propolis, inhibits Streptococcus mutans glucosyltransferase activity [32]. Our data showed that daily gargling with propolis solution reduced cariogenic microorganisms in plaque by nearly twofold after 14 days (Table 4). Machorowska-Pieniążek et al. evaluated propolis effects on dental plaque using the approximal plaque index, orthodontic plaque index, gingival index, and supragingival bacterial plaque [33]. Samples collected at baseline and after 35 days showed that ethanol-extracted propolis reduced the gingival index, orthodontic plaque index, and percentages of Actinomyces spp. and Capnocytophaga spp. compared to the control group [33]. The antimicrobial activity of propolis against Streptococcus mutans and Lactobacillus spp. has been confirmed by multiple studies [34, 35] and is supported by our findings (Table 4).

V. Conclusion:

This study demonstrated that xylitol lollipops, propolis mouthwash, and probiotic tablets significantly reduce cariogenic microorganisms in dental plaque among children aged 7–12 years, with varying degrees of efficacy. These findings suggest that xylitol lollipops are the most effective among the tested interventions for reducing plaque-associated cariogenic microorganisms, followed by propolis mouthwash, while probiotic tablets show selective efficacy against *Mutans Streptococci*. Incorporating these over-the-counter products into preventive oral health strategies could enhance caries control, particularly in high-risk populations.

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