

EFFECT OF PROBIOTIC MILK ON THE LEVELS OF *STREPTOCOCCUS MUTANS* IN CHILDREN: AN *IN-VIVO* CLINICAL STUDY

Paediatric

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ABSTRACT

Background: Dental caries is one of the most common diseases of childhood, characterized by an increased number of *S. mutans* in the mouth. Adaptation of Hippocrates "let food be your medicine" falls true for the probiotic foods. Probiotic bacteria are thought to reduce this infectious disease. Introduction of live microbial food supplement benefits the host and works as an alternative to eradicate the infection.

Aim and Objectives: The aim of this study is to evaluate the levels of *Streptococcus mutans* in the oral cavity of children, before and after the consumption of probiotic milk.

Materials and Method: In this cross-sectional study, 50 healthy, school going children, aged between 7-10 years, free from dental caries were selected. Evaluation was done and followed up for 5 days. Assessment of *S. mutans* count was done at baseline after one hour and at an intervention period of 5 days, of the probiotic and plain milk consumption, using blood agar.

Results: A statistically significant reduction in the *S. mutans* level was seen in children after the probiotic milk consumption.

KEYWORDS

Probiotic milk, dental caries, *Streptococcus mutans*, saliva.

INTRODUCTION

Dental caries is one of the most common diseases affecting the teeth of a child. A world-wide distribution of caries ranges from 60-90% in school children to nearly 100% in adults.¹ It is the most common and chronic microbial disease of complex etiology predominantly causing tooth loss. Reduction in dental caries is one of the objectives to achieve in the Global Goals 2020. However, it has shown increasing trend in the developing countries including India.²

Over the years, research studies established that *Streptococcus mutans* (MS) is one of the most common cause for dental caries in humans. It can rapidly metabolize dietary sugars to acid, reducing the pH.³ Research in the field of dental caries prevention has been focusing on ways for reducing or totally eradicating cariogenic flora from the oral cavity. However, most of the studies have shown that it is difficult to completely eliminate *Streptococcus mutans* from oral cavity by mechanical and chemical control.⁴ Dental caries could be prevented by targeting the pathogen directly using antimicrobial agents and indirectly by interfering with the ecological pressure of the pathogen.⁵ In an era where increasing health-care costs, rising food literacy, and the Hippocratic concept of "let food be thy medicine and medicine be thy food" has led to discovery of "functional foods".⁶ One of the novel methods for prevention of dental caries is by manipulation of resident oral microorganism by ingestion of probiotic organisms.⁷

The term 'probiotic' was first coined by Lilly and Stillwell, in the year 1965, meaning 'for life'.⁸ Food and Agriculture Organization/World Health Organization ((FAO/WHO) in 2001 defined it as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".⁹

Probiotics are live microorganisms that provide health benefits to the host when administered in proportionate amounts.¹⁰ There are reports on gastrointestinal and urogenital problems, allergic diseases, and to enhance the function of the digestive tract, thereby, promote the immune system.¹¹ The mechanism of probiotics in the oral cavity is not completely understood, but they reduce CFU counts of cariogenic pathogens.¹² Additionally, probiotics modulate both humoral and cellular inflammatory responses affecting lactic acid, hydrogen peroxide, and bacteriocins, which are antimicrobial agents produced by lactic acid bacteria.^{12,13,14} Most of the studies reviewed discuss the ability of the lactic acid to compete with pathogens for adhesion on surfaces and for nutrients, causing the displacement of the latter.¹²

Elimination of pathogens can be done by probiotic approach as they

survive in acidic environment of dental caries in the oral cavity.¹⁵ *Lactobacilli* and *bifidobacteria* are frequently used and studied probiotic organisms for replacement of cariogenic bacteria in probiotics.¹⁶ Dairy foods like cheese, yoghurt and milk are considered useful vehicles for probiotic bacteria.¹⁶ Hence the probiotic vehicle should be suitable for all ages and especially for young children.¹⁷

Limited studies have investigated the effect of milk containing probiotic bacteria in children and other dairy products.¹⁷ Since very little have been explored in this part, a cross-sectional study was planned and carried out in caries-free school children, aged 7-10 years, with the aim to evaluate the levels of *Streptococcus mutans* before and after the consumption of probiotic milk in their oral cavity.

The term probiotic is a Greek word which means "for life". According to the World Health Organization probiotics are live microorganisms when administered in adequate amounts, confers health benefit to the host.⁶

Ingestion of probiotics in food substances or in tablets and capsules, have benefitted health by reducing the incidence of dental caries, improved management of periodontitis, abridged halitosis and oral candidal infections.⁷

Hence, this study was carried out to assess the influence of probiotics in the form of milk, added to the daily diet of the study group to find its effect on the salivary levels of *Streptococcus mutans* at initial intervention and after a 5 days follow-up.

MATERIALS AND METHODS

This prospective study was planned and carried out in the Department of Paediatric Dentistry in association with Microbiology Department. The ethical clearance was obtained from the concerned authority. A signed consent was obtained from the children and the parents.

For this study, 50 subjects aged between 7-10 years, were randomly selected from Rajkiya Sr. Sec School, Debari, Udaipur, Rajasthan, India.

The commercially available probiotic fermented milk Yakult (supplied by Yakult UK Ltd.) was given to the subjects consecutively over a period of 5 days. Each bottle of 65ml Yakult fermented milk contained minimum of 6.5×10^9 viable cells of *Lactobacillus* species.⁸

Children in the age group of 7-10 years with no history of antibiotic

intake proceeding 1 month, no clinically detectable caries and absence of previous preventive dental treatments were included in the study and children with any congenital abnormalities, mental disorders, systemic disorders and undergoing orthodontic treatment were excluded.

The saliva samples of selected children were collected and kept for evaluation of the initial *S. mutans* count. These children were then randomly divided into 2 groups, each of 25. Subjects in Group A were provided with 65ml Yakult milk (Fig. 1) and that in Group B with plain milk for 5 days. All the subjects were encouraged to maintain good oral hygiene.



Fig.1 65ml Yakult milk

Following a clinical examination, the subjects in each group were asked to drink the Yakult or the plain milk. After 1 hour, they were asked to swallow the residual saliva. The saliva samples were then collected in sterile containers and labelled as A or B, according to the respective groups (Fig.2,3).



Fig.2 Saliva sample of Group A after the consumption of probiotic milk

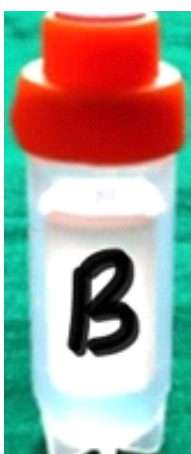


Fig.3 Saliva sample of Group B after the consumption of plain milk

For the purpose of analysis, the samples were handed over on the same

day to the Department of Microbiology, Pacific Institute of Medical Science, Udaipur city.

All plates were cultivated on mitis salivarius bacitracin agar medium at 37 °C under anaerobic conditions for 72 hours. Confirmation of *S. mutans* was performed under light microscopy after staining a heat fixed smear. Microbial counts were expressed as colony-forming units per ml of saliva.⁹ Saliva assessment was done before the consumption of the probiotic and plain milk, after 1 hour and on the 5th day. Colonies of *S. mutans* were identified on the basis of their morphology and counted using digital colony counter.

RESULT

In this study, mean *S. mutans* count before the consumption of probiotic milk and plain milk was found to be 2.63×10^5 , and after 1 hour was found to be 2.58×10^5 and 2.62×10^5 respectively. When compared after 5 days, there was a significant reduction in the *S. mutans* count in the subjects consuming probiotic milk. The mean count after 5 days for probiotic milk was 2.35×10^5 and that for plain milk was 2.60×10^5 . After the comparison of *S. mutans* count, at a duration of 1 hour and 5 days, a statistically significant reduction in *S. mutans* count was observed (Table.1, Fig.4)

Table 1. Mean *Streptococcus mutans* count at baseline, 1 hour and 5 days after probiotic milk and plain milk consumption (CFU/ml)

Experimental Groups	Yakult milk (65ml) Group A	Plain milk Group B
Baseline	2.63×10^5	2.63×10^5
After 1 hour	2.58×10^5	2.62×10^5
After 5 days	2.35×10^5	2.60×10^5

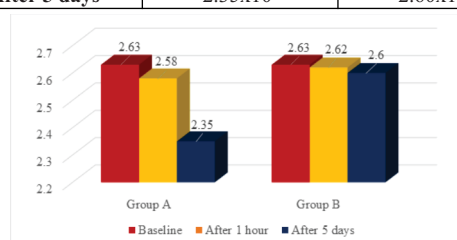


Fig. 4 Comparison of the variations in the *S. mutans* count at baseline, after 1 hour and after 5 days of the consumption of probiotic milk and plain milk ($\times 10^5$ CFU/ml)

DISCUSSION

With the variations in global trends, emergence of antibiotic resistant strains has become a problem now. It is a well-established fact that caries are characterized by colonization of the tooth surface biofilm (dental plaque) by *mutans streptococci*-in humans, while *S. mutans* have been implicated as specific organisms associated with initiation of caries.¹⁰ The ability of these microbes to adhere firmly to the surfaces of salivary protein-coated teeth and plaque biofilm and increase in number by both growth and recruitment from planktonic phase (salivary suspension) by auto-aggregation on exposure to dietary sucrose is an important event in caries formation and progression.¹¹

Villegas L M *et al.*,³ conducted a study on 363 pre-schoolers aged 3-4 years. They were supplied with 200 mL of milk with *Lactobacillus rhamnosus* 5×10^6 and *Bifidobacterium longum* 3×10^6 . After 9-months follow-up, the authors concluded that regular consumption of milk containing probiotic bacteria reduced the *Lactobacillus* sp and salivary buffering capacity but no significant difference was found in the prevalence of dental caries. Dakshinamoorthy M *et al.*,¹² used dark chocolate containing probiotic bacteria for their study and concluded that the probiotic bacteria had an inhibitory effect on the *Streptococcus mutans*. Tehrani MH *et al.*,¹³ stated that use of probiotic drops, decreased the *S. mutans* count without changing the count of *Lactobacillus* sp. Bafna HP *et al.*,¹ proved that *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 present in the Amul probiotic yogurt were effective in reducing the *S. mutans* levels in saliva. Ashwin D *et al.*,¹⁰ concluded that probiotic ice-cream can reduce caries causative organisms, but its long term or synergistic effect on oral health are still unknown.

The levels of salivary *S. mutans* found in the present study were statistically significant when compared after 1 hour to that after 5 days. A marked reduction in the cariogenic bacteria was found after the

consumption of probiotic milk due to possible mechanism of action of probiotics against the ability to early colonize in oral cavity, bacterial adhesion, immunomodulation and interspecies interaction.¹⁴ Grover and Luthra,¹⁵ reported that the probiotics interact directly and indirectly in the oral cavity. The possible direct interactions in dental plaque includes binding of oral microorganisms to protein, production of chemicals that inhibits oral bacteria, action on plaque formation and its complex ecosystem by competing and intervening with bacterial attachments. Various indirect probiotic actions are modulation of systemic immune function, enhances local immunity, regulates mucosal permeability and effects on the non-immunologic defence mechanism. It also functions as antioxidants and neutralizes free electrons thus prevents plaque formation.

In the current study, the effect of probiotic milk on the cariogenic flora was done only for a short duration. Though, the short-term administration of probiotic milk, resulted in the reduction of *S.mutans* counts in the oral cavity of caries-free children, still further studies are required to evaluate its long-term effects.

CONCLUSION

In accordance with the results, the present study concludes that, daily consumption of probiotic milk reduces the *S.mutans* count in the mouth of children by displacing the pathogenic bacteria. In order to prevent enamel demineralization, probiotic products could be used which provide a natural defense against the harmful bacteria in oral cavity. Though the inhibition of one strain only cannot be implied on the whole species of cariogenic flora.

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