



RESEARCH ARTICLE

ROLE OF PROBIOTICS IN CARIES CONTROL: A CLINICAL STUDY

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ABSTRACT

Objectives: To compare streptococcus mutans levels in saliva, before and after consumption of milk containing probiotic and probiotic curd.

Materials and Methods: Twenty caries free children in the aged 12-14 years were selected and divided into two groups A and B respectively. Children in group A was given probiotic curd for first 7 days and group B was given probiotic milk for first 7 days. 7 days wash out period was given to both the groups and for next 7 days, group A was given probiotic milk and group B was given ml probiotic curd. Saliva samples were assessed at baseline, 1 hour after consumption, on 7th day, after wash out period (14th day) and on 21th day.

Results: The study revealed a reduction in salivary mutans streptococci (MS) counts after 7 days in both the groups. After 7 days, probiotic milk showed a statistically significant reduction in MS counts. The difference in the reduction of MS counts with probiotic milk and probiotic curd at 21th day was not statistically significant.

Conclusion: The use of probiotic products could be an alternative strategy of displacing pathogenic microorganisms by probiotic bacteria

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INTRODUCTION

Dentists play a pivotal role in providing dietary guidance to children. The role of diet in health and well-being is universally acknowledged. With the evolution of the science of nutrition, research is now being directed towards improving the understanding of specific physiologic effects of the diet beyond pertaining to its nutritional value. The development of probiotics during the past decade has signalled an important advancement in the food industry which is leading to the development of various aspects of foods. The term probiotic, meaning "for life" is derived from the Greek language. The term probiotic refers to "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO report (2002) (Guarner *et al.*, 2005). The concept of probiotic evolved from Elie Metchnikoff's ideas that the bacteria in fermented products could compete with microbes that are injurious to host and are hence, beneficial to health. The mechanism of action of probiotics is the replacement of pathogenic species with non-pathogenic species (Anitha Chinnappa, 2013). Dairy foods such as cheese, yoghurt and milk are considered as substantial vehicles for probiotic bacteria.

The delivery is found to be suitable for all the age groups, especially for young children as it has been said that exposure in the early life may facilitate a permanent establishment of health promoting strains of probiotic. Hence, the present study was undertaken to investigate the effect of curd and skimmed milk containing probiotic bacteria on caries-associated microorganisms (mutans streptococci) levels in saliva and comparison of both as cariostatic food.

MATERIALS AND METHODS

Randomized controlled cross over study was carried out over a span of 21 days was conducted with a sample size of 20 children.

Inclusion criteria

Children in the age group of 12-14 years with all permanent teeth erupted (except 3rd molars),

Exclusion criteria

Children with clinically detectable caries. History of any preventive dental treatment Children on antibiotic medication Children undergoing orthodontic treatment and medically compromised children.

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The study was approved by ethical committee of the institute. A written consent was obtained from the parents and selected children were equally divided into two groups A and B respectively. Children in group A was given 100 ml of probiotic curd and group B was given 65 ml of probiotic milk curd respectively for a period of 7 days. Both milk and curd was given once daily preferably in the morning. Baseline 1 ml unstimulated saliva sample was taken from each participant 1 hour before the consumption and after 7 days in the sterile container containing transporting media. Then 7 days wash out period was taken. After wash out period crossover was done. Group A was given 65 ml of probiotic milk while group B was given 100 ml of probiotic curd for next seven days. Baseline (B2) saliva sample taken was before crossover and after crossover. Salivary streptococcus mutans colony counting (CFU) was done using Mitis salivarius Bacitracin agar. The number of colonies was counted and subjected to paired and unpaired t-test statistical analysis.

Table 1. Comparison of baseline scores with 7th day (Curd) for Group A using paired t test (before cross over)

Group A	No of participants	Mean (SD)
Baseline	10	120225.60
7 th day	10	100074.90 (7305.7)
t value	-	5.311
P value	-	<0.001**

Table 2. Comparison of baseline scores with 7th day (milk) for Group B using paired t test (before cross over)

Group B	No of participants	Mean (SD)
Baseline	10	124888.20
7 th day	10	82123.80
t value	-	9.971
P value	-	<0.001**

Table 3. Comparison of Group A and Group B using unpaired t test (before cross over)

7 th day scores	No of participants	Mean (SD)
Group A	10	100074.90 (7305.7)
Group B	10	82123.80 (10898.2)
t value	-	4.327
P value	-	<0.001**

Table 4. Comparison of baseline scores with for Group A (milk) using paired t test (after cross over)

Group A	No of participants	Mean (SD)
Baseline	10	124761.90 (7035.2)
7 th day	10	88267.80 (7311.1)
t value	-	22.871
P value	-	<0.001**

Table 5. Comparison of baseline scores with 7th day (Curd) for Group B using paired t test (after cross over)

Group B	No of participants	Mean (SD)
Baseline	10	104154.90 (3037.1)
7 th day	10	93566.50 (4953.5)
t value	-	11.140
P value	-	<0.001**

RESULTS

Before cross over both group A and group B showed significant (<0.001) reduction in salivary streptococcus mutans count (Table 1 and Table 2) compared to baseline values.

Table 6. Comparison of baseline scores of Group A and Group B using unpaired t test (after cross over- B2)

Baseline scores	No of participants	Mean (SD)
Group A	10	124761.90 (7035.2)
Group B	10	104154.90 (3037.1)
t value	-	8.504
P value	-	<0.001**

Table 7. Comparison of Group A and Group B using unpaired t test (after cross over)

7 th day scores	No of participants	Mean (SD)
Group A	10	88267.80 (7311.1)
Group B	10	93566.50 (4953.5)
t value	-	1.897
P value	-	0.074

In table 3 Group B shows statistically significant ($P < 0.001$) reduction in MS counts as compared to Group A. After cross over, both group A and group B show significant (<0.001) reduction in salivary Mutans streptococcus count compared to baseline values. Results achieved after cross over comparing group A and group B showed in Table 7 were not statistically significant.

DISCUSSION

Traditionally, probiotics have been associated with gut health, and most clinical interest has focused on the prevention or treatment of gastrointestinal infections and diseases; however, during the last decade, an increasing number of established and proposed health effects of probiotic bacteria have been reported, including enhancement of the adaptive immune response, treatment or prevention of urogenital and respiratory tract infections, and prevention or alleviation of allergies and atopic diseases in infants (Anna Haukioja, 2010). The concept of oral foci of infection, affecting the systemic health has been extensively researched and removal of these oral foci using probiotics in the oral cavity has dictated tremendous improvement in general as well as oral health of the individual. This proposed concept is reinforced by recent evidences.

Table 1 and 2 revealed a significant reduction in the salivary mutans levels with probiotic milk and curd over 7 days period, compared to baseline. The possible mechanism of action of probiotics include prevention of adhesion of pathogens to host tissues, Stimulation and modulation of the mucosal immune system, Modulation of cell proliferation and apoptosis through cell responses, Improvement of intestinal barrier integrity and up regulation of mucin production, Killing or inhibition of growth of pathogens through production of bacteriocins or other products, such as acid or peroxide, which are antagonistic towards pathogenic bacteria, Involvement in binding of oral microorganisms to proteins (biofilm formation), Action on plaque formation and on its complex ecosystem by competing and intervening with bacteria-to-bacteria attachments Involvement in metabolism of substrates (competing with oral microorganisms of substrates available) (Devine, 2009; Lima, 2005). Though both groups showed significant results, group B results in approximately 34% reduction in salivary mutans count compared to group A (16%). A minimum level of 10^6 CFU/g of probiotic strain should be present in food products to confer the benefits of probiotics. Probiotic milk used in this study contains 6.5 billion of Lactobacillus casei strain Shirota, while the probiotic curd used in the study contained L. acidophilous strain

(100gm⁻¹ billion) and was manufactured by Nestle India, Pvt. limited. After cross over it was seen that Group A showed 29% reduction and Group B showed 10% reduction in the salivary mutans count. An in vitro study done by Lima *et al* states that there was no significant difference in adhesion of *L.acidophilus* and *L.caseii* strains to artificial caries model (Lima, 2005). However, in the present study significant values were achieved because of the varied Probiotic content in yakultand probiotic curd. Also, current evidence indicates that probiotic effects are strain-specific; therefore, a beneficial effect attributed to one strain cannot be assumed to be provided by another strain, even when it belongs to the same species (Senok, 2005). Baseline salivary samples taken just before the crossover after the wash out period as presented in table 6 showed significant difference due to insufficient wash out period. After corss over Group A and Group B shown in table 7 showed that there is no significant difference in reduction of salivary mutans count because of the inadequate wash out period due to which the effect of probiotic milk used was considered to remain in the oral cavity. However it is important to state that the salivary level of caries-associated microbes does not equate to dental caries. In fact, the microbiota of unstimulated whole saliva resembles that of the tongue more than of dental plaque.

Conclusion

This study hence concludes that probiotic has considerable effect in reducing the microbial load of caries associated microbes. However, further studies with a larger sample size and substantiality of probiotics are necessary to conduct. There is further scope of studies to evaluate the effect of probiotics on active caries.

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