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Original Article

Dentistry Section

Effect of Probiotic Curd on Salivary pH and *Streptococcus mutans*: A Double Blind Parallel Randomized Controlled Trial

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ABSTRACT

Background: Dairy products like curd seem to be the most natural way to ingest probiotics which can reduce *Streptococcus mutans* level and also increase salivary pH thereby reducing the dental caries risk.

Objectives: To estimate the role of probiotic curd on salivary pH and *Streptococcus mutans* count, over a period of 7 days.

Materials and Methods: This double blind parallel randomized clinical trial was conducted at the institution with 60 caries free volunteers belonging to the age group of 20-25 years who were randomly allocated into two groups. Test Group consisted of 30 subjects who consumed 100ml of probiotic curd daily for seven days while an equal numbered Control Group were given 100ml of regular curd for seven days. Saliva samples were assessed at baseline, after ½ hour 1 hour and 7 days of intervention period using pH meter and Mitis Salivarius Bacitracin agar to estimate salivary pH and *S. mutans* count. Data was statistically analysed using Paired and Unpaired t-test.

Results: The study revealed a reduction in salivary pH after ½ hour and 1 hour in both the groups. However after 7 days, normal curd showed a statistically significant ($p< 0.05$) reduction in salivary pH while probiotic curd showed a statistically significant ($p< 0.05$) increase in salivary pH. Similarly with regard to *S. mutans* colony counts probiotic curd showed statistically significant reduction ($p< 0.05$) as compared to normal curd.

Conclusion: Short-term consumption of probiotic curds showed marked salivary pH elevation and reduction of salivary *S. mutans* counts and thus can be exploited for the prevention of enamel demineralization as a long-term remedy keeping in mind its cost effectiveness.

Keywords: *Bifidobacteria*, Dental caries, *Lactobacillus*

INTRODUCTION

The term probiotic, which literally means "for life" was first coined in the 1960s by Lilly and Stillwell [1]. The Food and Agriculture Organization (FAO) and World Health Organization (WHO) defined probiotics as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' [1]. The idea behind probiotics is the replacement of pathogenic species with non-pathogenic types such as strains of *lactobacilli* or *bifidobacteria*, which are the most common types of microbes used as probiotics [2].

A number of probiotic induced benefits on the general health have been proposed, such as reduced susceptibility to infections, reduction of allergies and lactose intolerance, as well as lowered blood pressure and serum cholesterol values [3]. However, data are still sparse on the probiotic action in the oral cavity.

There is variety of commercially available probiotic products containing different bacterial strains such as lozenges, sucking tablets, chewing gums and dairy products such as milk, ice cream, cheese, yoghurt, etc [4]. Among these, dairy products are the most natural and useful vehicles, but an ideal administration vehicle has yet to be identified [5]. In this context, curd is an interesting probiotic food, as it is popular and universally liked.

Probiotics play a role in creating better oral health through its direct and indirect interactions. Through its direct interactions, probiotics interfere with biofilm formation, compete with oral microorganisms for available substance and produce chemicals to inhibit oral harmful bacteria that damage the oral hygiene. Through indirect interactions, probiotics play role in removing harmful bacteria and stabilizing normal conditions, modulating systemic immune function on local community as well as non-immunologic defence mechanisms [6].

Probiotics have the ability to lower the salivary pH and produce antioxidants which utilize the free electrons required for mineralization of plaque thereby inhibiting plaque formation [7]. Inhibiting the colonization of *S.mutans* on the tooth surface, is believed to prevent the formation of dental plaque and development of dental caries [8]. The literature review indicated that there are hardly any interventional studies relating the role of probiotics in the oral cavity. Hence present study is undertaken to assess the effect of probiotic curd on salivary pH and *Streptococcus mutans* count.

MATERIALS AND METHODS

Study design: This double blind parallel randomized clinical trial was carried out in the Department of Public Health Dentistry, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow from April 6, 2015 for a period of 7 days. Ethical clearance was obtained from the institutional ethical committee and informed consent was taken from all the participants of the study.

Study population: All the nursing volunteers in the age range of 20-25 years of Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow were asked to participate in the study and those who accepted were included as per the following inclusion and exclusion criteria [Table/Fig-1].

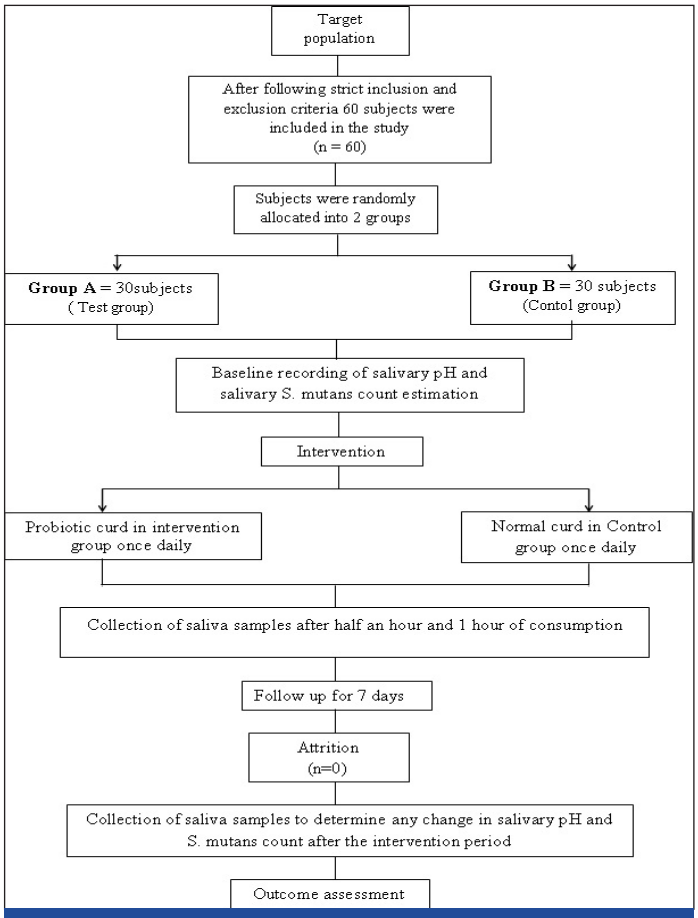
Inclusion criteria

1. Subjects aged between 20-25 years.
2. Subjects with no active carious lesions.
3. Healthy non-smokers, without any systemic diseases.
4. Subjects with no history of any preventive dental treatment.

Exclusion criteria

1. Lactose intolerant subjects.
2. Those undergoing antibiotic treatment during the course of the study.

3. Those who were on any other probiotic supplements during the course of the study.
4. Subjects on use of xylitol products for last three weeks and during the course of study.
5. Subjects with a history of intra-oral surgery within the last 6 months.



[Table/Fig-1]: Flow diagram of study protocol according to CONSORT

STUDY PROCEDURE

Sample size calculation was based on the proportion of reduction in colony counts as observed in pilot study.

The sample size (n) was calculated from the following formula:

$$n=\frac{(z_\alpha)^2 pq}{L^2}$$

where

z_α=1.96 (assuming the distribution is normal and confidence limit is 95%)

p= The proportion of reduction in colony counts as observed in pilot study (in %) =80

q=1-p (in %) =20

L= Permissible error in estimation of p= 20% of p (standardized) = 20% of 80=16

Substituting the values in the formula

$$\text{Sample size (n)} = \frac{(1.96)^2 \times 80 \times 20}{(16)^2}$$

$$= 24$$

The number of subjects per group is approximately 24 thus a total sample size of 48 subjects were included in the study.

Based on the above calculations, a sample size of 30 subjects per group was used in this study considering dropouts (attrition) thus totalling to 60 subjects.

Randomization

Accordingly 60 subjects both male and female were included and randomly allocated into 2 groups of 30 each.

Group A- Test (Probiotic curd) group.

Group B- Control (Normal Curd) group.

Random sequence generation was done by using computer generated table of random numbers by independent investigator and sequential allocation was done by the investigator. The allocation sequence was concealed from primary investigator in sequentially numbered, opaque, sealed and stapled envelops. Accordingly the primary investigator allocated the subjects as per the sequentially numbered envelops to the test group and control group [Table/Fig-1].

Blinding: In the study both the primary investigator and the subjects were blinded. The probiotic curd (Mother dairy b-activ Plus®) and the normal curd (Mother dairy®) both had similar colour, taste and consistency and were dispensed in identical cups measuring 100ml and was coded as 'A' and 'B' by an independent investigator. The subjects were then instructed to consume curd daily in the morning before breakfast at the venue for 7 days.

INVESTIGATION PROCEDURE

Saliva sampling: Sampling of stimulated whole saliva was carried out between 9 a.m. to 10 a.m. in the morning before breakfast at baseline and after ½ hour, 1 hour and 7 days of intervention. After thorough rinsing with water, the subjects were asked to chew on a piece of paraffin wax for 5 minutes and the pooled saliva was collected directly into 30ml of sterile plastic container. Saliva samples from each subject was collected in two separate containers both at the baseline and after 7 days of intervention for testing salivary pH and *Streptococcus mutans* count estimation while rest of the samples were collected in single containers. The saliva samples of all the patients were identified by a code number.

Salivary pH Test: All the saliva samples were tested on the same day for salivary pH at 20° C using pocket sized digital pH meter (pHep®, Hanna instruments, Singapore). The equipment was calibrated with distilled water to reach pH 7 everytime before each sample was measured.

Salivary *Streptococcus mutans* count estimation: Saliva samples of baseline and after 7 days of intervention were tested for *Streptococcus mutans* count estimation. The samples were transported to the laboratory within 4 hours in order to preserve the viability of probiotic bacteria and processed on the same day. The sample was vortexed for 15 seconds using Cyclomixer, CM 101®. Approximately 1 µL of saliva sample was inoculated on the dry Mitis Salivarius Agar with potassium tellurite medium and bacitracin. The plates were incubated at 37°C in 5-10% CO_2 jar for 48 hours. After 48 hours, colony characteristics were studied and the number of Colony Forming Units (CFU/ml) of *Mutans Streptococci* in saliva was determined.

STATISTICAL ANALYSIS

Data were collected by single calibrated examiner (Kappa=0.83). In the present study there was no loss to follow up. The data gathered was entered in the proforma and was subjected to statistical analysis. The results were analysed using the Statistical Package for Social Sciences System (SPSS) version 17.0. Paired t-test was employed for comparison between before and after intervention values of the same group and Student t-test for comparison between test and control group.

RESULTS

In the test group the study results showed a highly significant reduction in the mean salivary pH from baseline to ½ hour and one hour of consumption and a highly significant increase in the

Time Interval	Test group	Control group	Mean difference	t	p-value
	Mean±SD	Mean± SD			
Baseline	7.35±0.42	7.37±0.44	-0.02	-0.1203	0.9046
½ hour	7.04±0.45	6.97±0.45	0.07	0.6043	0.5480
1 hour	7.29±0.44	7.18±0.44	0.11	0.9383	0.3520
7 days	7.58±0.40	7.28±0.44	0.30	2.8136	0.0067*

[Table/Fig-2]: Comparison of test and control group with respect to salivary pH levels at baseline, ½ hour, 1 hour and 7 days of consumption
*p<0.05, * Statistically significant

	Probiotic curd	Normal curd	Mean difference	t	p-value
	Mean± SD	Mean± SD			
Baseline	4.46±0.30	4.48±0.27	-0.02	-0.3673	0.7147
After 7 days of consumption	4.24±0.38	4.47±0.27	-0.27	-2.6594	0.0101*

[Table/Fig-3]: Comparison of test and control group with respect to log salivary *streptococcus mutans* counts at baseline and 7 days of consumption
*p<0.05, * Statistically significant

mean salivary pH from baseline to 7 days (p=0.00001). The control group showed a highly significant reduction in the mean salivary pH from baseline to ½ hour, one hour and 7 days of consumption (p=0.00001) [Table/Fig-2].

On comparative evaluation between the two groups after 7 days of consumption the mean pH value in test group was higher compared to the control group and this difference was statistically significant [Table/Fig-2].

The test group showed a highly significant reduction in *streptococcus mutans* count from baseline to 7 days of consumption than control group [Table/Fig-3].

However, after 7 days of consumption the mean number of colonies was found to be higher in control group as compared to test group and this difference was found to be highly significant (p=0.0101) [Table/Fig-3].

DISCUSSION

Dental caries is a multifactorial disease of bacterial origin which often requires a multimodal approach for control and treatment. The use of probiotics enriched products such as milk, ice cream, lozenges, cheese and yoghurt has been one of such approaches [4]. Several of these normal dietary constituents contain microorganisms, which can function as probiotic agents. However, these specially formulated probiotic products contain around 10^{10} to 10^{11} colony forming units of microorganisms per millilitre [9].

Probiotics incorporated into dairy products neutralize acidic conditions in the mouth and interfere with cariogenic bacteria [8]. Ferrazzano et al., suggested that the vehicle for administration of probiotics should be of milk origin due to contained casein phosphopeptides (CPPs) that have inhibitory effect on demineralization and promote the remineralization of dental enamel [10]. Hence the present study was conducted with an aim to investigate the inhibitory effect of consumption of probiotic curd containing *Lactobacillus* acidophilus on salivary pH and salivary *Streptococcus mutans* count and to compare this with normal curd in caries free nursing volunteers.

Curd (Mother dairy b-activ Plus® and Mother dairy®) was selected as it is a common dietary constituent of the Indian population, is readily available, provided with full of nutrients and can be used in several different combinations that are acceptable to all age groups. The common brand which was available in Lucknow was chosen. Unlike milk and milk products, curd being semisolid may be retained in the oral cavity for a longer period of time with an extended beneficial effect.

The findings of the present study revealed a significant increase in salivary pH and significant reduction in salivary *streptococcus mutans* count from baseline to 7 days with probiotic curd, as compared to normal curd. The findings of the study were similar to the study conducted by Chinnappa et al., who also showed a statistically significant reduction in *Streptococcus mutans* count as compared to control curd and control ice-cream over a period of 7 days [1].

Another study conducted by Hoorizad et al., also compared the effect of probiotic yoghurt and non-probiotic yoghurt on the salivary pH of orthodontic patients and concluded that probiotic yogurt can cause an increase in the salivary pH providing a suitable condition for remineralization of tooth mineral structures [11].

In the present study the decrease in salivary pH on consumption of normal curd could be due to the acidic nature of the curd. Inspite of this decrease, the pH levels were still above the critical pH (5.2-5.5), which is always considered as harmful for the causation or progression of dental caries. This indicates that curd though acidic in nature does not pose any risk for caries [4]. However, consumption of probiotic curd resulted in an increase in salivary pH thus proving it to be more beneficial than normal curd.

The findings of the present study are in contrast to the study done by Sudhir et al., who showed a reduction in the salivary pH after 30 days of consumption which was statistically significant in the probiotic group but not in the normal curd group [4].

Streptococcus mutans has been implicated as specific organism associated with initiation of caries. Therefore, the effect of probiotic curd containing *Lactobacillus* acidophilus on *S. mutans* count was looked into [4].

With regard to *streptococcus mutans* count the seventh day result showed that there was statistically significant reduction with probiotic curd as compared to normal curd. This could be due to the fact that probiotic micro-organisms present in probiotic curd does not ferment lactose and sucrose and are temporarily able to colonize the oral cavity for upto 2 weeks thereby preventing the colonization of *S. mutans* on tooth surface [8]. This was similar to the study done by Sudhir et al who showed a statistically significant reduction in *S. mutans* count in probiotic group after 30 days time interval [4]. Parallel studies have been done which also suggest that consumption of products containing probiotic *lactobacilli* or *bifidobacteria* could reduce the number of *Mutans Streptococci* in saliva [3,12,13].

Lactobacillus and *Bifidobacteria* the most widely used bacterial strains that have been explored for their potential probiotic properties [1]. The focus is on these species as they are already produced in the dairy industry and are rarely implicated in any human infections [1]. In fact these bacteria show symbiotic relationship with humans. They are present in the mucous membrane on the epithelial cells of the gut where they inhibit the growth and attachment of harmful bacteria by producing bactericidal chemicals against these bacteria. With the development of evidences regarding usefulness and safety of probiotics, these bacteria are replacing the traditional prophylactic and treatment regimes [14]. There is a concept where these 'beneficial' microorganisms can inhabit a biofilm and actually protect oral tissue from diseases. It is possible that one of these biofilm's mechanism to keep pathogens out is to occupy a space that might otherwise be occupied by a pathogen [15]. Probiotics should adhere to dental tissues to establish a cariostatic effect and thus should be a part of the biofilm to fight the cariogenic bacteria [16]. Comelli et al., selected and examined 23 micro-organisms with potential probiotic properties for prevention of dental caries and identified that two *Streptococcus thermophilus* and two *Lactococcus lactis* strains were able to adhere to saliva coated hydroxyapatite beads to the same extent as *Streptococcus sobrinus* and thus concluded that such a property in a non- pathogenic dairy bacterial strains can help to decrease the cariogenic potential of dental plaque [17].

In the present study, probiotic curd were also accepted well by all the participants similar to normal curd which suggest that Probiotic curd can serve as an appropriate vehicle for administration of probiotics in everyday life. Inspite of the salivary pH testing and *S. mutans* count estimation not being the major way of assessing the effect of probiotic, but it gave the practical result indicating that probiotic can be used for prevention of dental caries. In the present study the only source of potential bias might be that we have not standardized the oral hygiene status of all the participants. This could have been avoided by performing oral prophylaxis of all the participants at the baseline. Present study findings can be generalized by taking larger sample size and with longer follow up period.

CONCLUSION

Short-term daily ingestion of probiotics delivered via curd in diet, showed marked salivary pH elevation and reduction of salivary *S. mutans* counts. Thus it can be suggested that probiotic dairy products can be adopted as a novel approach for prevention of enamel demineralization as a long-term remedy.

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