

THE EFFECTS OF PROBIOTICS ON THE SALIVARY *STREPTOCOCCUS MUTANS* AND *LACTOBACILLI* LEVELS AMONG PRESCHOOL CHILDREN: A CLINICAL TRIAL

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ABSTRACT

Background: Prevention of dental caries plays a vital role in dental public health practice. *Streptococcus mutans* is considered to be the chief pathogen in caries development. One of caries preventive methods is probiotics which can interfere with bacterial colonization, compete with oral microorganisms.

Aim: To evaluate the effects of probiotics in yogurts on the salivary *Streptococcus mutans* and *Lactobacilli* colonies count among preschool children aged (3 – 6 years).

Materials and Methods: A controlled clinical trial was conducted on (350) children who were divided into two groups, control and study group. The control group instructed to ingest one cup of regular yogurt and the study group ingest yogurt with probiotics for two weeks. Salivary *Streptococcus mutans* and *Lactobacilli* levels were counted at the baseline and after one week and two weeks of usage. One way ANOVA test, unpaired t test and Tukey post hoc test were used to compare between the groups

Results: A statistically significant reduction in salivary *Streptococcus mutans* colonies count were seen with in the study group compared with the control group.

Conclusion: Probiotics showed maximum decrease in the *Streptococcus mutans* colonies count after short period of usage.

INTRODUCTION

Tooth decay is the most common chronic disease of early childhood, it is a multi-factorial infectious and transmissible disease which can be prevented^[1].

The most acceptable theory of caries development is the chemico-parasitic theory which is based on

the presence of cariogenic bacteria, susceptible host and fermentable carbohydrate; therefore, chemical and mechanical microbial plaque control are recommended^[2,3].

Streptococci mutans are the major pathogenic bacteria in the caries process. This group

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mainly includes *Streptococcus mutans* (SM) and *Streptococcus sobrinus*, which are responsible for caries development in both animals and humans^[4]. *Streptococcus sobrinus* is the primary bacterial pathogen of smooth surface dental caries. It presents in 43-60% of plaque cultures of children with early childhood caries^[5]. It is supposed that the inhibition of these microorganisms leads to caries prevention. Inhibiting the colonization of SM on the tooth surface is believed to prevent the formation of dental plaque and development of dental caries^[6].

World Health Organization (WHO) defined probiotics as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host^[7]. The concept of probiotics is based on 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 in the control of dental caries^[8].

Probiotics can improve oral health through its direct and indirect roles. In the direct interactions, probiotics interfere with bacterial colonization, compete with oral microorganisms for available substrates and produce chemicals to inhibit oral harmful bacteria that damage the oral hygiene. However in 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 defense mechanisms^[9]. Probiotics can reduce the salivary pH and produce antioxidants which utilize the free electrons required for mineralization of plaque thereby inhibiting plaque formation^[10].

Probiotics are available in a variety of commercial products like lozenges, sucking tablets, chewing gums and dairy products such as milk, ice cream, cheese and yoghurt^[11]. Among these, dairy products are the most natural and useful vehicles^[12]. Yoghurt and other fermented milk-based products are beneficial for general health, because of their probiotic content. They were proposed to be used

as an alternative to manage many disorders such as infectious diseases, cancers and gastrointestinal problems in particular^[13].

Several studies concluded that probiotics affect oral health including prevention of dental caries^[14-16]. Taipale et al^[16,17] conducted two studies to verify the effect of the early administration of probiotics (*Bifidobacterium animalis* subsp. lactis BB-12) on oral colonization of *Streptococci mutans* of 106 infants from ages of 1-2 months to the age of 2-years. At the first evaluation children showed a lower SM colonization percentage. After that the same children were re-evaluated at the age of 4 years to assess SM level in their plaque and the presence of dental caries in their deciduous teeth, there were no differences among groups concerning both parameters.

Juneja et al^[18], and Lexner et al^[19], evaluated the effect of milk containing *Lactobacillus rhamnosus* (hct 70 or LB21) for few weeks among small group of adolescents. They did not find significant differences in SM count in subjects who received milk with probiotics compared to subjects received milk without probiotic.

Aminabadi et al^[20], evaluated the effect of yogurt containing *Lactobacillus rhamnosus* GG as a probiotics vehicle for three weeks on 105 children, there was a significant decrease in SM count immediately after probiotics use alone, but recolonization was described during the five consecutive weeks. Cildir et al^[21] carried out a study on 24 healthy adolescents, undergoing orthodontic treatment, to assess the effect of yogurt containing *Bifidobacterium animalis* subsp. Statistical significant reduction of SM was recorded after probiotics yogurt consumption.

Several studies investigated the effect of probiotics strain on *Lactobacilli* level, but they did not find significant change in *Lactobacilli* counts in saliva and/or plaque^[16,19,22]. Another two studies evaluated the probiotics effect on caries lesion development^[22,23]. A statistically significant

difference in caries increment was recorded only in one paper in subjects who received probiotics and fluoride compared to subjects who received placebo milk [23].

According to the findings of some studies that have illustrated the role of probiotics in reduction of cariogenic micro-flora of the mouth, this study aimed at; comparing the effect of probiotics containing yogurt and probiotics free yogurt on *Streptococcus mutans* and *Lactobacilli* salivary levels.

MATERIAL AND METHODS:

This was a randomized clinical trial study to estimate the effect of probiotic on *Streptococcus mutans*, *Lactobacillus* counts.

Subject:

The study was conducted on preschool children with an age (3-6 years), selected from kindergartens in Damietta city. Children who were the (d) component was (0) according to dmft index (WHO, 1962) [24], had systemic diseases or were taken antibiotics within one month before the study were excluded.

Sample size:

The sample is calculated by (<https://www.surveysystem.com/sscalc.htm>). The confidence level (95%) and confidence interval (5%) with accessible population (1600) were used. the calculated size was (310).

Educational part:

To ensure maximum cooperation from parents and their children, before starting the study health educational lectures were conducted by the two examiners to all children and their parents in the selected kindergartens about the dental caries, how to prevent and the role of probiotics in caries prevention. Standardized oral hygiene practices were explained to the children and reinforced at every visit.

Clinical part:

Ethical approval from the ethical committee at Mansoura University was obtained. The approvals from the kindergarten's authorities and informed consents from children parents were obtained before starting the study. From examined (800) child of both genders only (372) were included. The final number of children who's their parents was signed the informed consents and approved to participate was (350). The sample was divided into control and study groups with an equal number (175) in each group. Control group included (86 male and 89 female children) while, the study group included (88 male and 87 female children). Two types of yogurt were used in this study, regular one and probiotics containing yogurts (Activia) contains billions of the probiotic culture *Bifidobacterium animalis lactis* DN-173 010/CNCM I-2494.

Both groups were instructed to consume one cup of yogurt (115 g) in the morning at the kindergarten time under supervision of the study examiners, or under their parents' supervision in the weekend.

Salivary sample collection:

Salivary samples were collected at the baseline and after one and two weeks from the yoghurt using. Children were instructed not to eat or drink at least one hour before sampling. The saliva was collected between 8 am to 10 am, children were asked to chew a piece of paraffin wax. The stimulated saliva was collected by suction method using sterile disposable syringes. One ml of collected saliva was put into test tube containing 1ml Reduced Transport Fluid (RTF) and sent for further microbiological procedure.

Microbiological Procedure:

The saliva samples were diluted in 0.05 M phosphate buffer to the dilution of 1/10 and agitated for 30 seconds on vortex mixer. 100 µl of the dilution was inoculated each on the Mitis Salivarius Agar with potassium tellurite medium and bacitracin and other 100 µl was inoculated on Rogosa Agar. Then all dishes incubated at 37°C in 5%-10%

CO₂ jar for 48 hours. After 48 hours, the number of colony forming units was determined using stereomicroscope, Labomed SZ 790, (Labomed, Inc. Los Angeles, CA). The colony count was expressed as the number of CFUs per milliliter (CFU/ml) of saliva by adjusting for the dilution factor. To ensure the intra-observer consistency, the colony counting was done by the same observer under the same conditions.

Statistical analysis:

Statistical analysis was carried out using SPSS Version 17.0 (Statistical Product and Service Solutions, IBM Corporation, New York, NY, USA). Results were expressed as percentages and chi-square was used to compare between groups. One way ANOVA test, post Hoc Tukey test and t test were used to compare between different means. P value equal or less than 0.05 is considered significant.

RESULTS

The total mean dmft scores and its components were estimated for each child in the both control and study group, no significant differences were found regarding the age, total dmft mean scores or its components. (Table 1)

Regarding the mean CFUs of *Streptococcus mutans* multiplied by 10⁶ at the baseline for the control and study group showed no significant difference (p = 0.09). On the other hand, after one week and two weeks, significant differences were found by comparing the two groups. Control group

used the regular yogurts, showed no significant change in the number of *Streptococcus mutans* CFUs along the study period (p = 0.06). While, the study group used the probiotics containing yogurts showed a significant decrease in the number of *Streptococcus mutans* CFUs along the study period (p = 0.01). (Table 2)

Table (3) showing the mean number of CFUs X 10⁶ of *Lactobacilli* among the control and study groups, the mean level among control group who used regular yogurts was nearly the same with that for study group at the baseline. After one week of yogurts usage the mean level of *Lactobacilli* tend to had significant increase among the study group compared with control one (p = 0.05). After two weeks, non-significant increase in *Lactobacilli* level among study group compared with control group (p= 0.08). No significant change in the mean *Lactobacilli* CFUs number was notice among the control group during the study period (p= 0.06), similarly in the study group non - significant increase in the mean *Lactobacilli* CFUs was noticed after one week and to weeks of probiotics yogurts usage (p= 0.06).

The pairwise comparison using post Hoc, Tukey test was shown in (table 4), control group showed no significant differences regarding the *Streptococcus mutans* and *Lactobacilli* mean CFUs levels. On the other hand, probiotics yogurts usage group showed significant decrease in the *Streptococcus mutans* level and slight increase of *Lactobacilli* levels after one week and two weeks of usage.

TABLE (1) The mean and standard deviation of age, total dmft and its components for both control and study groups

	Control	Study	t (p value)
	Mean ±SD	Mean ±SD	
Age	4.620 ± 0.323	4.821 ± 0.571	0.924 (0.908)
dt	2.314 ± 0.742	3.011 ± 0.839	1.835 (0.136)
mt	0.521 ± 0.031	0.382 ± 0.063	0.896 (0.922)
ft	1.723 ± 0.099	2.114 ± 0.182	2.397(0.09)
dmft	4.558 ± 0.977	5.507 ± 1.014	2.994 (0.06)

SD: standard deviation

*: Significant at p level ≤ 0.05

TABLE (2) the means and standard deviations of *Streptococcus mutans* level (CFUs x 10⁶) among control and study groups at baseline and after one week and two weeks of yogurt usage.

	At baseline	After one week	After two weeks	F (p value)
	Mean ±SD	Mean ±SD	Mean ±SD	
Control (Regular yogurt)	215.12 ± 20.214	210.31± 21.312	217.37± 19.997	2.379 (0.06)
Study (Probiotics yogurt)	211.89 ± 18.632	115.21± 9.784	77.94 ± 4.862	7.742 (0.01)*
t (p value)	1.764 (0.09)	4.025 (0.05)*	6.941 (0.01)*	

SD: standard deviation

F: one way ANOVA test

t. unpaired t test

**: Significant at p level ≤ 0.05*

TABLE (3) The means and standard deviations of *Lactobacilli* level (CFUs x 10⁶) among control and study groups at baseline and after one week and two weeks of yogurt usage.

	At baseline	After one week	After two weeks	F (p value)
	Mean ±SD	Mean ±SD	Mean ±SD	
Control (Regular yogurt)	90.29± 9.832	88.98± 9.996	90.12±8.972	2.093 (0.06)
Study (Probiotics yogurt)	89.97 ± 9.773	92.28± 10.324	93.53± 12.832	2.423 (0.07)
t (p value)	0.928 (0.889)	2.968 (0.08)	2.289 (0.06)	

SD: standard deviation

F: one way ANOVA test

t. unpaired t test

**: Significant at p level ≤ 0.05*

TABLE (4) p values of the pairwise comparison for the control and study groups regarding the *streptococcus mutans* and *lactobacilli* levels using post Hoc Tukey test

Pairwise comparison	<i>Streptococcus mutans</i>		<i>Lactobacilli</i>	
	Control	Study	Control	Study
Baseline vs one week	0.935	0.05*	0.869	0.09
Baseline vs two weeks	0.09	0.01**	0.975	0.09
One week vs two weeks	0.989	0.01**	0.09	0.06

**: Significant at p level ≤ 0.05*

****: Significant at p level ≤ 0.01*

DISCUSSION

Probiotics incorporated into dairy products neutralize acidic conditions in the mouth and interfere with cariogenic bacteria^[6]. Ferrazzano et al^[25], demonstrated that the vehicle for administration of probiotics should be of milk origin as it contains casein phosphopeptides (CPPs) that have inhibitory effect on demineralization and promote the remineralization of dental enamel. Using yogurt (more than or up to 4 times a week) might be associated with a lower incidence of dental caries in children^[26].

The type of microorganism that was used in this study was *Bifidobacteria*. It is known that *Lactobacillus* and *Bifidobacteria* are the most widely used bacterial strains that have been known for their potential probiotic properties and already produced in the dairy industry and are rarely implicated in any human infections^[6].

The findings of this study revealed that yogurt containing probiotics (*Bifidobacterium animalis lactis*) significantly reduced the number of *streptococcus mutans*. Several studies have been done and suggested that consumption of products containing probiotic *Lactobacilli* or *Bifidobacteria* could reduce the number of *Streptococci mutans* in saliva^[21,27,28].

Also SrivaStava et al^[29], showed high significant reduction in *Streptococcus mutans* count from baseline to 7 days of consumption of curd containing probiotics than control group. In addition to that Sudhir et al^[11], demonstrated a statistically significant reduction in *Streptococcus mutans* count in probiotic group after 30 days.

These results could be attributed to the action of probiotic micro-organisms which does not ferment lactose and sucrose and are temporarily able to colonize the oral cavity for up to 2 weeks thereby preventing the colonization of *Streptococcus mutans* on tooth surface^[6]. Also probiotics create a biofilm

which act as a protective lining for tissues and keep pathogen away with unfavorable environment for pathogens^[30].

However regarding the effect of yogurt containing probiotics on *Lactobacilli* level, there was non-significant increase in *Lactobacilli* level after one and two weeks. These finding was agreed with Sidhu et al^[31], who demonstrated an increase in *lactobacillus* count in 67% of their study subjects, also Ahola et al^[28], reported an increase in *Lactobacilli* count after intake of probiotic in the type of cheese over a period of three weeks. Caglar et al^[32], and Singh et al^[33], showed that probiotic could decrease the count of *Streptococcus mutans*, but its effect on *Lactobacilli* count has not been significant. This finding could be attributed to the *Lactobacilli* content of the probiotics which could partially participate to the higher *Lactobacilli* count level after probiotics consumption^[6].

However our results did not match with Nozari et al^[34], who reported no effect of probiotics containing *Bifidobacterium lactis* on *Streptococcus mutans* or *Lactobacilli* levels. The difference between our study findings and that of Nozari could be attributed to the difference in the age range between both studies.

A systematic review carried out 2013^[35], to review the effect of probiotic on caries prevention concluded that, all studies performed on probiotics were of small and medium sample sizes and of short duration. More investigations are recommended on large scale and for longer durations.

The present study recommended the use of milk derivative with probiotics specially in children in conjunction with good oral hygiene measure for further protection from caries

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