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Evaluation of *Lactobacillus reuteri* probiotic Lozenge intake on Salivary Cariogenic Bacterial Counts in Preschool Children: A Randomized Clinical Trial

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RESEARCH ARTICLE

General Note

Article is recommended to print as color digital version in recycled paper.

ABSTRACT

Introduction: The current study was conducted to assess whether intake of probiotic *L. reuteri* lozenges on a daily basis is beneficial to combat caries-associated salivary bacteria, namely Mutans streptococci and lactobacilli, hence minimizing dental plaque accumulation while keeping the buffer capacity of saliva to optimum levels in a group of otherwise healthy preschool children. *Materials and Methods:* The study group comprised of 178 healthy children (aged 3 - 6 years). The *L. reuteri* probiotic lozenges were given to experimental group (n=90) while the control group (n=88) received placebo lozenges, two times per day for a total duration of 56 days. Chair-side caries risk test sets were used to count salivary amounts of mutans streptococci, lactobacilli and also assess the buffering capacity. Simplified Oral Hygiene index (OHI-S) was employed to qualify dental plaque accumulation once at the startas a baseline and finally after 56 days. *Results:* The results showed a statistically significant reduction of *S. mutans* and lactobacilli in the experimental group versus baseline counts and the control group after 56 days (p=0.000 and p=0.035) respectively. Both the experimental and the control groups also showcased fewer plaque accumulations when compared with their baseline scores. While buffer capacity in experimental group was observed to be more than in the control group, the results reflected no statistically significant difference between the two study groups (p=0.576). The compliance was reported to be 80% and no adverse events were observed during the entire duration of the study. *Conclusion and Recommendations:* The regular intake of probiotic lozenge containing *L. reuteri* significantly lowered caries-associated bacterial counts and hence, probiotics consumption could aid in the reduction or even the prevention of dental caries.

Keywords: *L. reuteri, S. mutans,* probiotic, dental caries

1. INTRODUCTION

During the childhood period, dental caries is considered one of the most chronic conditions. It can have a severe and deleterious effect on the individual's wellbeing and social life. However, with all the recent innovations and advances in the field of conservative dentistry, prevention is on the horizon (Featherstone, 2000). In the past; the focus was mainly concentrated on contemporary factors such as host, dietary, and plaque biofilm removal in an attempt to prevent dental caries (Pitts, 2004; Marsh, 2004; Cooney, 2010). Recently, probiotic therapy was suggested as a substitute plan for caries prevention. They have surfaced as a natural and unconditional remedy in the resistance to infectious diseases through a shift from a pathogenic organism to a non-pathogenic or at least a commensal one (Chen and Wang 2010). Following the World Health Organization/Food and Agriculture Organization of the United Nations report (2002), "probiotics" are the live microorganisms that boost health if given in an appropriate amount (Guarner et al., 2005).

Studies involving the pediatric population and the use of probiotics have been variable. Some studies have shown a reduction in *Mutans Streptococci*, while others studies showed no decline. This could be attributed to the variability of methods adopted in those studies. For example, a small sample size or a short duration of time could have led to such differences (Aminabadi et al., 2011, Jindal et al., 2011; Cildir et al., 2012; Sudhir et al., 2012). Thus, more thorough research is needed to provide evidence base date for the use of probiotics in the childhood population.

The purpose of the study was at assessing whether the intake of probiotic *L. reuteri* lozenges on a daily basis can affect salivary bacteria "*Mutans streptococci and lactobacilli*" which associated with dental caries as well as the accumulation of dental plaque in the same time maintain the salivary buffer capacity in a group of healthy preschool children. The null hypothesis was that this daily intake of probiotic *L. reuteri* lozenges would have no effect on the mutans streptococci and lactobacilli the counts, dental plaque accumulation, or salivary buffer capacity.

2. MATERIALS AND METHODS

The research protocol was permitted by the Research Ethics Committee, Faculty of Dentistry, King Abdul-Aziz University's (proposal no. 031-41) & Clinical trial number NCT01601145.

According to the studies that were conducted before (Ahola et al., 2002; Çaglar et al., 2006), it was assumed that there would be a 30% reduction of Streptococcus mutans in the experimental group subjects and in 10% of the controls. For sample size calculation, the free web- based operating system Open Epi, version 3 was employed (Dean and Soe, 2009). To show a statistically significant difference amid the experimental and control groups; eighty subjects were required for a 5% significance level with 80% power.

Parents were handed out a fully detailed leaflet, with the aim of the study explained along with informed consent. The children were brought in for an initial clinical evaluation (375 subjects) after the written consent was obtained. The inclusion criteria were as follows: healthy children with no history of any systemic condition, having full set of primary dentition, high counts of salivary *S. mutans* (≥ 105 CFU) at their baseline, dmft score ≥ 1, and no history of recent antibiotics intake. Children with a history of medical conditions or systemic antibiotic or topical fluoride treatments 4 weeks before the baseline, except the fluoride in the toothpaste were not enrolled, nor were children who used xylitol chewing gums and dairy probiotics. After placement of all these factors, 178 children (ages 3 - 6 years) were suitable to take part in the study.

Study Lozenges

Contents of the lozenges for the research have at least 200 million live *L. reuteri*", *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 "(*L. reuteri* Prodentis®) developed by Biogaia in Stockholm, Sweden. It was made sure that both the placebo and probiotic lozenges were identical in shape, taste and size such that they could not be differentiated from each other. The probiotic and placebo lozenges were analogous in size, shape and taste and could not be specified. It is to be noted however, that the placebo lozenges had no probiotic bacteria. White plastic bottles were used to pack the lozenges with written instructions on how to use them. Both the examiners and the contributors were unable to identify which were the probiotic lozenges and which were the placebo ones.

Study design

The study conducted adopted a double-blind randomized placebo-controlled design. Using graphpad.com, a random number generator was employed to carry out the randomization such that a list of random numbers was generated at the end. Blinding was employed for all the study personnel, laboratory technicians and involved clinicians. The duration of intervention was 56 days with a questionnaire filled at the baseline. This questionnaire involved inquiries about the characteristics of each subject at the start of the research. Group A (Experimental group, n = 90) took probiotic lozenges containing atleast 200 million live *L. reuteri* (*L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 (*L. reuteri* `Prodentis®) for 56 days and Group B (Control group, n= 88) received placebo lozenges with no probiotics. Instructions were as follows; parents were asked to give the lozenges to their children for two times a day while making sure that they were taken at the same time each day; once in the morning and the other in the evening followed by tooth brushing. Regular oral hygiene measures were strongly advocated applying a "pea-size" amount of fluoridated toothpaste for two times a day (AAPD, 2014). The parents were given both the tooth brushes and tooth paste. Any adverse side effects from the daily intake of the lozenges were asked to be reported by the parents right away.

Clinical examination

Following both the inter-examiner and intra-examiner calibration, two pediatric dentists were employed to carry out the intraoral examinations. According to the modified WHO criteria for diagnosis of dental caries; decayed, missed and filled teeth (dmft) index was used to measure the Dental caries status at the baseline (WHO, 1997). Bite wing radiographs were also employed for each child to view proximal caries. Using a modified version of simplified Oral Hygiene index (OHI-S), with amendments to fit the primary dentition, plaque accumulation was scored (Miglani et al., 1973). This was performed at the baseline (T0) and then after 56 days (T1) for every child.

Salivary Microbial and buffer capacity evaluation

According to the manufacturer's instructions; CRT® (IvoclarVivadent AG, Bendererstrasse 2, FL-9494 Schaan, Liechtenstein) was employed to measure the salivary mutans streptococci, lactobacilli counts, and buffer capacity. Scores of "low" (less than 105 CFU/ml) or "high" (greater than 105 CFU/ml) were given for the salivary mutans streptococci and lactobacilli counts as per manufacturer recommendations. One investigator was employed for all the readings using the model chart. As for the buffer capacity; the scores were "high BC" which was assigned a blue color, "medium BC" which was assigned a green colour and "low BC" which was assigned a yellow color. All the analysis of the salivary mutans streptococci, buffer capacity and lactobacilli counts were also done at baseline (T0) and after 56 days (T1).

Compliance assessment

The parents were received A compliance Calendar to record the lozenges ingestion daily by their children by marking each box for the corresponding lozenge. That was performed to check and ensure compliance. For extra assurance, the remaining lozenges that were given back by each parent were counted to assess any missed intake of lozenges. The parents/guardians were received a text message as a reminder on a weekly basis.

Statistical methods

The Statistical-Package-for-Social-Sciences or SPSS (version 20 Inc., Chicago, IL, USA) software was utilized to process the data acquired. Mean and standard deviation were used for the description of this data. Frequencies and percentages were used to describe the qualitative measurements. Cronbach's Alpha was used to calculate internal consistency. Inter-class correlation (ICC) test was employed to perform inter-examiner and intra-examiner reliability. Group comparison among lozenges groups in quantities variables was assessed using the t-test while the Chi square test was utilized for the qualitative variables. Using the Wilcoxon signed-rank test, differences in the inter-group of salivary buffer capacity, salivary lactobacilli counts and salivary *S. mutans* counts were evaluated. P-value < 0.05 was selected to measure the statistical significance.

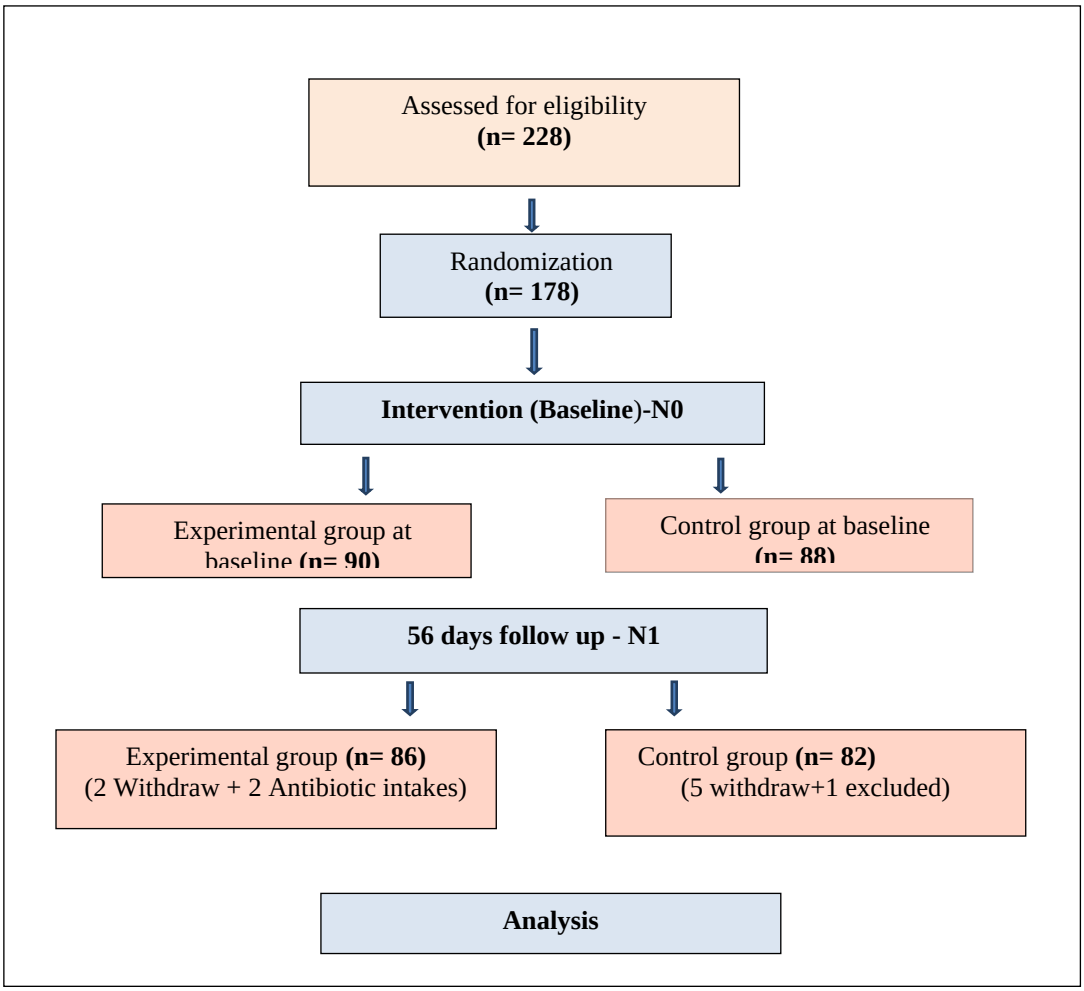

Figure 1 Flow diagram showing the number of subjects in experimental and control groups at the beginning and completion of the clinical trial.

3. RESULTS

Intra-class correlation was employed to measure intra-examiner reliability for each examiner for recording dmft, and it was 0.920 and 0.934 respectively. While inter-examiner reliability, was 0.965 implying an almost excellent level of agreement. Inter-examiner reliability was 0.819 for debris component (DI-S) of simplified Oral Hygiene index (OHI-S) measurement, implying a strong level of

agreement. Using CRT® test, the intra-rater reliability for caries-associated salivary bacterial counts (*Streptococcus mutans* and *Lactobacillus*) was (0.83) as regard to the kappa test of agreement (figure 1). Table 1 shows baseline characteristics of the children who were selected in the study. Table 2 shows the subjects' salivary parameters and OHI-S values. Compared to the baseline counts and the control group for both salivary mutans streptococci and lactobacilli, the experimental group demonstrated significant reductions. However, there was no significant statistical difference regarding the buffer capacity records among the study groups at the baseline (p= 0.151). At the end of the period of intervention, there was also no statistically significant difference regarding the buffer capacity records between the experimental and the control groups (p= 0.576). However, all the groups showed statistically significant decrease in the debris component (DI-S) of (OHI-S) score. Lozenge consumption compliance was recorded to be over 80% by the parents indicating a perfect compliance. Between the experimental and control groups, the ratio of participants with perfect compliance to those with less than perfect compliance showed no statistically significant differences in the ratio (p = 0.822) as highlighted in table 3. No adverse side effects were reported in any group (figure 2 – 6).

Table 1 Baseline characteristics of children who entered the study

Baseline characteristic		Experimental n = 90 (100%)	Control n =88 (100%)	Total n =178 (100%)	p value*
Gender%	Male	38 (42.2%)	32 (36.4%)	70(39.32 %)	0.446
	Female	52(57.8%)	56 (63.6%)	108(60.68 %)	
Mean Age (Years)		4.90 (±1.012)	4.98 (±0.950)		0.617
Mean dmft at baseline		6.83± 2.94	7.28± 3.728		0.353

* Statistically significant p-value<0.05, using Chi-Square test

Table 2 Percent distribution of salivary mutans streptococci counts, lactobacilli counts, buffer capacity and mean debris component (DI-S) of (OHI-S) score between the experimental and control groups

point of time	Baseline			After the intervention		
Groups	Experimental n1 (%)	Control n2 (%)	p value*	Experimental n1 (%)	Control n2(%)	p value*
Mutans streptococci (cfu/mL)						
High (≥10^5)	90 (100)	88 (100)	NA	39(45.3)	64(78)	0.000*
Low (<10^5)	0 (0)	0 (0)		47(54.7)	18(22)	
Lactobacilli (cfu/mL)						
High (≥10^5)	75 (83.3)	83.3 (77)	0.526	57(66.3)	67(81.7)	0.035*
Low (<10^5)	15 (16.7)	16.7 (11)		29(33.7)	15(18.3)	
Buffer capacity						
High	45 (50)	50 (45)	0.151	61(70.9)	53(64.6)	0.576
Medium	27 (30)	30 (34)		19(22.1)	20(24.4)	
Low	18 (20)	20 (9)		6(7)	9(11)	
Debris component (DI-S) of (OHI-S)						
mean ± S.D	1.6 (±0.4)	1.6 (±0.49)	0.979	0.774(±0.333)	0.915(±0.489)	0.010*

* Statistically significant p-value<0.05, using Chi-Square test.

Table 3 Compliance (112 lozenges) in the experimental and control groups

Number of Lozenges	Experimental group n = 90 (%)	Control group n = 88 (%)	P-value*
Perfect = 112	73(81.1)	70(79.54)	0.822
Less than 112	17(18.9)	18 (20.45)	

* Statistically significant p-value<0.05, using Chi-Square test.

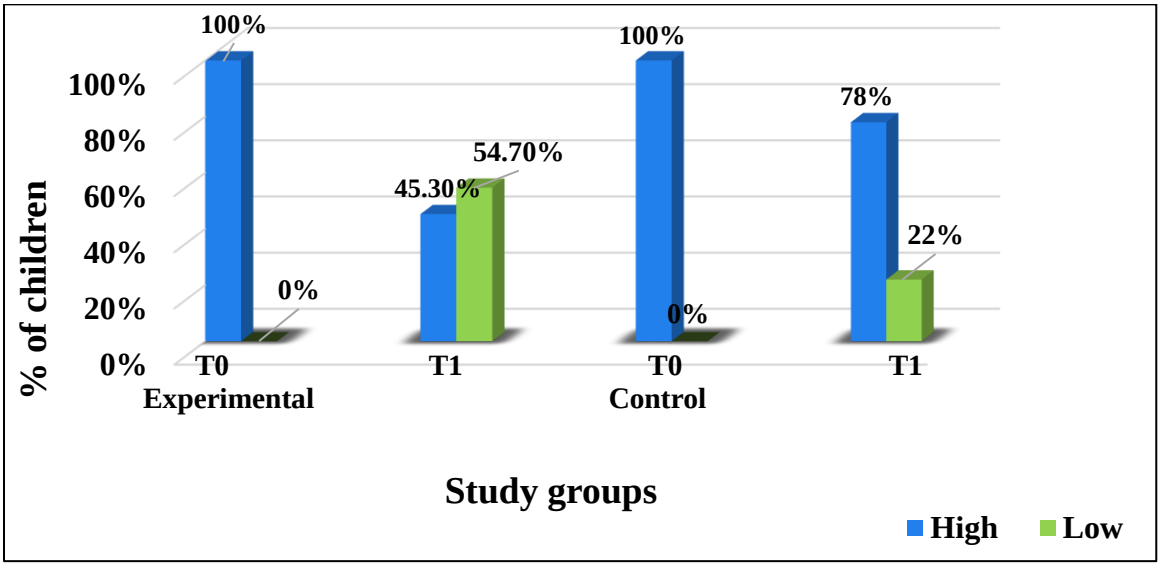

Figure 2 Percentage distribution of children according to salivary mutans streptococci counts in the study groups at different time point. T0: base line, T1: after 56 days

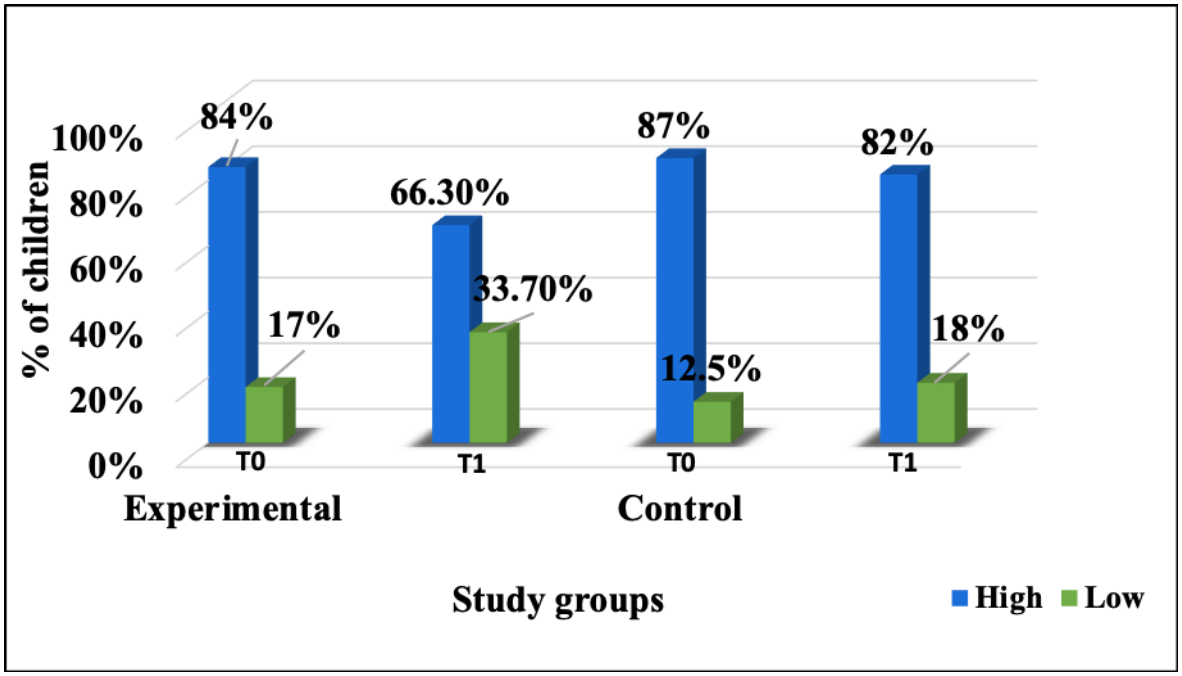

Figure 3 Percentage distribution of children according to salivary lactobacilli counts in the study groups at different time point. T0: base line, T1: after 56 days

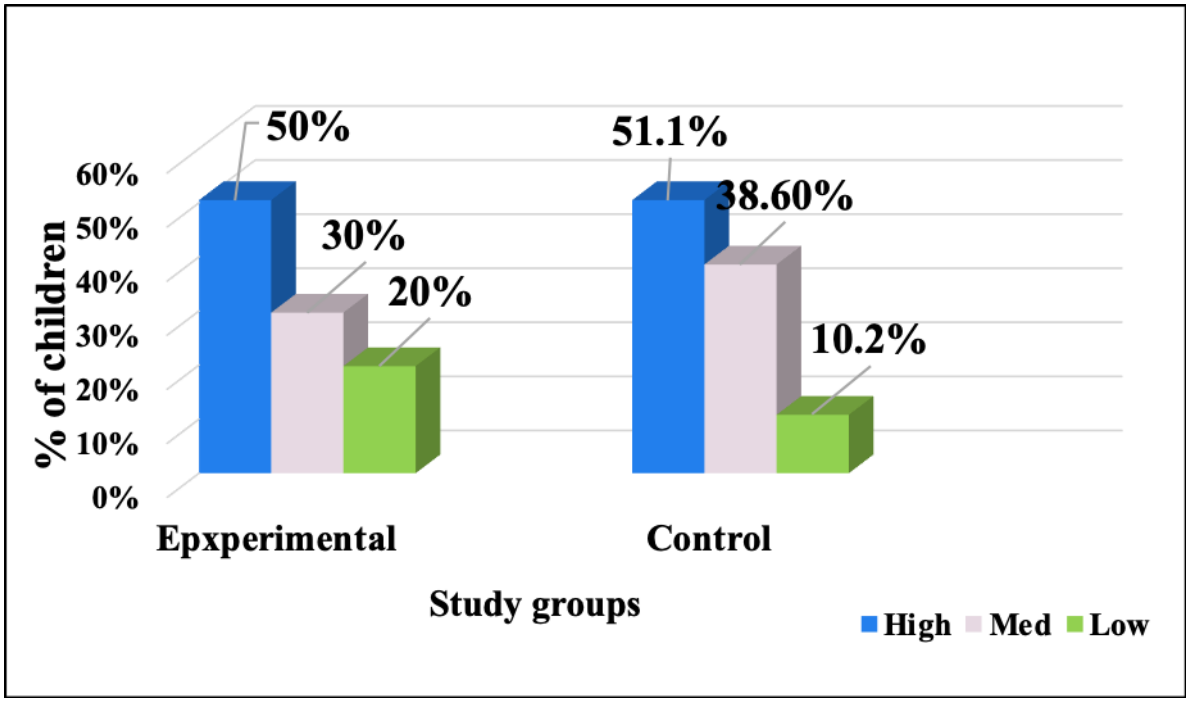

Figure 4 Percentage distribution of children according to salivary buffer capacity in the study groups at the baseline.

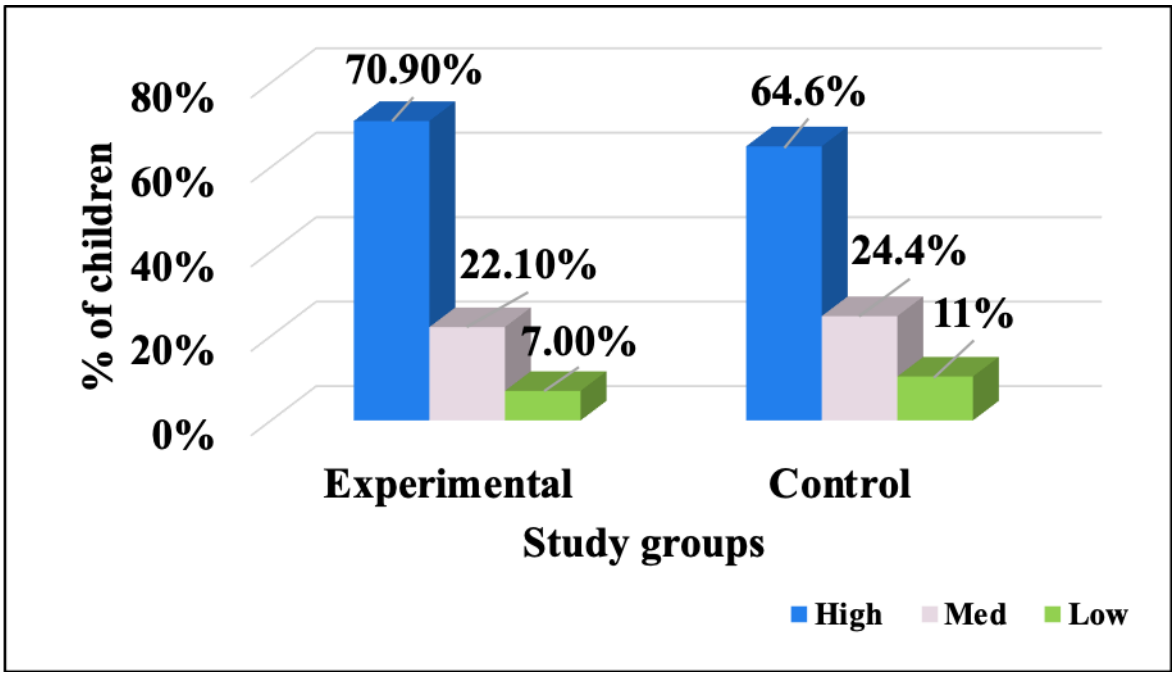

Figure 5 Percentage distribution of children according to salivary buffer capacity in the study groups after 56 days.

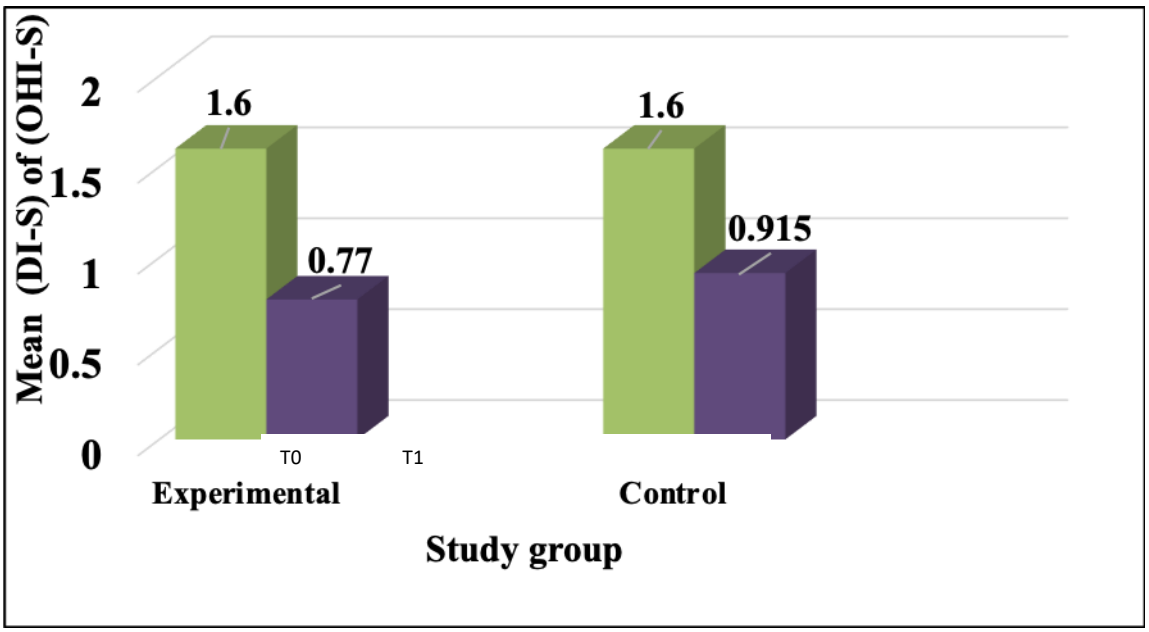

Figure 6 Comparison of mean of debris component (DI-S) of (OHI-S) score in each study group at different time point of the trial. T0: base line, T1: after 56 days

4. DISCUSSION

Dental caries poses a serious financial burden around the world, especially in Saudi Arabia. This data is in accordance with the fact that the prevalence of dental caries is roughly estimated to be around 80% for the primary dentition, using a mean dmft of 5.0 and 70% for children's primary dentition, using a mean dmft of 3.5(Al Agili, 2013). Several attempts have been tried to try and cut back on its prevalence and deleterious effects. One of these is whether the ingestion of probiotic *L. reuteri* lozenges daily is successful in decreasing caries-associated counts of salivary bacteria and accumulation of dental plaque, and adjusting salivary buffer capacity, in a high caries risk group, as, healthy preschool children in Jeddah, Saudi Arabia. Currently, all the dental literature does not support enough clinical studies conducted on the use of oral probiotics in the children population. The present study is one of the very scarce clinical studies implemented to investigate the effectiveness of *Lactobacilli reuteri* probiotic therapy in the form of lozenges on salivary bacterial counts, *Streptococcus mutans* and Lactobacillus, also known as caries-associated bacteria in preschool children and then to evaluate dental plaque accumulation and the effect this will have on buffer capacity of saliva in children.

Based on their caries experience (dmft) and salivary mutans streptococci levels, the study group were identified as a high-risk group. The focus on this group was to target those entities who are at a specially elevated risk of cultivating dental caries in the future. Not to mention that changes in mutans streptococci counts can be readily identified and effectively compared between the two study groups further making choosing this group an added advantage. Concerning baseline characteristics, dental caries (dmft), frequency of tooth brushing, intake of fluoride supplements and dietary habits, there were no intergroup differences. Also, there was no statistically significant difference found indicating the homogeneity of the sample in all aspects. Based on the results of this study, it was discovered that mutans streptococci counts were significantly decreased in the experimental group as opposed to the control group by the end of the intervention period of 56 days. This could be attributed to the release of the antimicrobial compound, reuterin, by *L. reuteri* which could be in part be responsible for this positive effect (Ashwin et al., 2015).

Debris component (DI-S) of (OHI-S) score observed at the end of the intervention phase of 56 days between the experimental group and the control group showed a significant difference indicating that the technique employed during this study was efficient for the reduction in dental plaque accumulation. This discovery was in accordance with other studies. These studies realized that children who utilised either a probiotic or chlorhexidine mouth rinses had reduced build-up of plaque compared with the control group (Harini and Anegundi 2010). Moreover, a study was done to explore the "on the counter" combined formulation of a probiotic for its effect on plaque, gingivitis and salivary *Streptococcus mutans* levels in subjects with chronic gingivitis and it revealed comparable results (Dhawan and Dhawan, 2013). They found a statistically significant reduction in plaque index (PI) in the probiotic group as opposed to control one. Also, a study carried out by Toiviainen et al. (2015) discovered that the use of lozenges consisting

of a combination of *Lactobacillus rhamnosus* GG and *Bifido bacterium animalis* subsp. lactis BB-12 lowered the plaque index (PI) in the probiotic group.

In divergence from our results, Sinkiewicz et al. (2010) uncovered that regarding the plaque index, there was no significant change in probiotic group. However, the control group illustrated a statistically significant increase in plaque index. The author of the pervious study advocated that *L. reuteri* may negatively affect plaque build-up even though no significant effect in the probiotic group could be developed. Another aspect that was considered by this study was how *L. reuteri* probiotic therapy could affect salivary counts of lactobacilli. It was found that there was statistically significant decline in Lactobacillus level in the experimental group when compared to the control group. This finding was in coherence with other studies. One of these studies is the study conducted by Cogulu et al. (2010). It found a significant drop in both salivary mutans streptococci and lactobacilli after multi strain probiotic-kefir consumption. Another study by Çaglar et al. (2005) also discovered a decline of lactobacilli after probiotic use; however, this decline was not found to be statistically significant. In this study, the decrease in Lactobacillus levels showed in the experimental group may have been owing to the enhanced oral hygiene measures for children by their parents.

The buffer capacity value was evaluated in this study. The current study showed that the capacity was not lowered from "high" to "low" or "medium" in any of the cases in either group. As a matter of fact; the children who began the study with "low" or "medium" buffer capacity showed an elevation in the buffer capacity while those who started off with "high" values maintained these values. Although the buffer capacity in the experimental group was elevated more than in the control group, the results were not enough to suggest any statistical difference, and this implied that the fluctuations in buffer capacity might be because of the enhancement in OHI-S and not related to probiotic therapy alone.

To measure plaque accumulation, the debris component of the OHI-S, which is being accepted for measuring oral hygiene in extensive epidemiological studies, was chosen (Broadbent et al., 2011). The study demonstrated that both experimental and control groups experienced an advancement in the debris component of the OHI-S by the end of the 56 days intervention period. This enhancement in the group of preschool children in the control group could be linked to the commonly known "Hawthorne effect" (which is also known as observer influence) (McCarney et al., 2007). This effect describes how the improvement in oral hygiene and plaque control may be due to the impact of being observed in the study rather than actual improvement. Guardians or parents in this study were specifically told that they were taking part in a trial concerning probiotic lozenges in an effort to lower cariogenic bacteria found in the saliva and plaque levels of their little ones. Hence, the knowing about the intended forthcoming of the study may have in one way or another led these parents to make sure that oral hygiene measures were strictly implemented at home.

This study was employed to prove that *L. reuteri* probiotic lozenges can be safely used with ease in children. The lozenge would also provide an acceptable vehicle for the administration of the probiotic. This was indicated by the high compliance level of both the parents and their children during the study protocol. However, the present study presented us with a few inevitable inherent limitations. First and foremost were the confounding variables. Randomization was attempted to dilute the effect of this evenly among the study groups; however, there was a possibility that they were not the same. Among these variables were food, fluoridated toothpaste, and the frequency of intake of food. It cannot be denied that these variables may exert a positive impact on the both the levels of cariogenic bacteria and plaque (Mejàre et al., 2014). Owing to the fact that the incidence of dental caries would necessitate a long-term follow up period which will add to the costs of further studies, another limitation would be the short term parameter of this study.

The current study was merely a drop in the ocean. An attempt to give an idea of how the implementation of oral probiotics can help in the management and thereby the deterrence of the benign epidemic currently knows as dental caries. It is instrumental to perform further studies to try and determine the optimum dose and time required for administration of each strain and how it could be delivered effectively for maximum prevention. Endeavors should be made to expand the knowledge and the perception of dental professionals with the idea of probiotics as an appendage to the dental caries prevention strategy. The ideal position that the dental professionals are in would help in the guidance of their patients toward well sought prophylactic and medicinal uses of probiotics that convey the anticipated positive oral health outcomes.

5. CONCLUSION

To conclude; caries-associated bacterial counts can be significantly reduced with the consumption of a probiotic lozenge containing *L. reuteri*. This consumption can concomitantly help in lowering the accumulation of plaque and hence encourage a better saliva buffer potential. However, more clinical studies are required to certify how and for how long the probiotic should be administered.

Conflict of interest

The authors did not have any conflict of interest related to this study.

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Abbreviations

MS	Mutans Streptococci
LB	lactobacillus
Dmft	decayed, missing and filled teeth of primary teeth
OHI-S	simplified Oral Hygiene index
DI-S	debris component of simplified Oral Hygiene index
AAPD	American Academy of Pediatric Dentistry
(T0)	At baseline
(T1)	After 56 days
ICC	Intra-class correlation
WHO	World Health Organization
BC	Buffer capacity
CRT	Caries risk test

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