ORIGINAL ARTICLE

Effectiveness of probiotic lozenges and Chlorhexidine mouthwash on plaque index, salivary pH, and Streptococcus mutans count among school children in Makkah, Saudi Arabia

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Abstract  Purpose: To compare the effect of the probiotic lozenges and chlorhexidine (CHX) mouthwash on plaque index (PI), salivary pH and Streptococcus mutans (S. mutans) 3 count among groups of Saudi children.

Methods: A total of 54 participants aged 8-12 years were randomly allocated into three groups, 18 children in each group. Children in the probiotic group consumed one probiotic lozenge (Biogaia prodentis) daily, while children in the CHX group were instructed to use CHX mouthwash twice daily. The control group was only instructed to follow regular oral hygiene measures. Saliva samples were taken at baseline, 15th and 30th days. PI scores, salivary pH values and S. mutans count were evaluated. Data were statistically analyzed using the ANOVA and the Tukey post-hoc test.

Results: Probiotic lozenges and CHX mouthwash significantly reduced PI and S. mutans count and increased the salivary pH values. However, there were no statistical differences between the
1. Introduction

Dental caries is a common chronic disease affecting children (Peres et al., 2019). The factors involved in the caries process include cariogenic microorganisms and, the tooth surface, and time. Dental caries, if left untreated, continues to form cavities on tooth surfaces, which are painful and infectious (Ng and Ramos-Gomez, 2012; Smith and Riedford, 2013).

Dental plaque is mainly responsible for caries. Brushing teeth and other mechanical plaque removal procedures can most effectively control plaque, apart from chemical plaque control agents. However, chemical agents show many side effects and unfavorable compliance (Nadkerny et al., 2015).

Among cariogenic microorganisms, *Streptococcus mutans* (*S. mutans*), which has many virulent factors, such as producing acids from carbohydrates, tolerating extreme acidic environments, and producing extracellular polysaccharides, which facilitate their adherence to another bacteria and the tooth surface (Caglar et al., 2008; Martinez et al., 2015).

Chlorhexidine (CHX) is an antimicrobial agent adsorbed onto the cell walls of the microorganisms. It has bacteriostatic and bactericidal effects (McDonnell and Russell, 1999; Eden, 2017). CHX varnish, gel, and mouthwash reportedly reduce the salivary *S. mutans* level, inhibit plaque formation, and increase salivary pH values (Jothika et al., 2015; Srivastava et al., 2016). However, its prolonged use can cause discoloration of teeth, altered taste, numbness, and drug resistance (Gizligoz et al., 2020).

Probiotics are live microorganisms, safe for human consumption, and have favorable effects on general health when consumed in adequate amounts (Pineiro et al., 2008; Mahantesha et al., 2015). Probiotics adhere to the tooth surface and compete with the cariogenic microorganisms and consequently inhibit their colonization and growth (Comelli et al., 2002).

However, probiotics are not particularly beneficial when it comes to oral health (Villavicencio et al., 2018). Moreover, probiotic therapy can overcome drawbacks of chemical products (Kamalaksharappa et al., 2018). Hence, this study aimed to compare the effectiveness of probiotic lozenges (BioGaia prodentis) and CHX mouthwash on plaque index (PI), salivary pH, and *S. mutans* count.

2. Materials and methods

2.1. Ethical statement

The proposal of this in vivo clinical trial was reviewed and ethically approved by the Institutional Review Board of the College of Dentistry, Umm Al-Qura University, with an IRB number (105–18). In addition to obtaining permission from parents, a written informed consent from the parents and children were obtained.

2.2. Study participants

Children participating in this were recruited from the Pediatric Dentistry Clinic of the College of Dentistry, Umm Al-Qura University, Makkah.

**G*Power (Version 3.1.9.2, Released 2014, Kiel University, Germany) was used to estimate the sample size, considering the alpha error left at 5%, the effect size to be measured (d) at 80%, and the statistical power of the study at 85%. The calculated sample size was 51 participants. Besides, one participant was added in each group for withdrawal probabilities.

Fifty-four children aged 8–12 years were randomly grouped into control, probiotic, and CHX (Clorasept) groups, with 18 participants in each group. A list of random numbers was created by a random number generator on www.randomizer.com.

The participants were selected based on the following inclusion criteria: children residing in Makkah, with a def and/or DMFT score ≥ 1, free from any systemic condition, who didn’t receive corticosteroids, systemic antibiotics, or any medication for at least 4 weeks before the study.

Eligible participants were scheduled for the next appointments to obtain saliva samples and detailed oral examinations. Parents were instructed to refrain the child from eating, drinking (except water), or brushing their teeth and flossing for 2 h prior to the sample collection.

2.3. Stimulated whole saliva samples

The participants were instructed to rinse their mouths with water to remove food residue before collecting the saliva samples. The stimulated whole saliva was collected by chewing a paraffin wax tablet for five minutes. The stimulated saliva was collected in a sterile polypropylene tube up to a volume of 5 ml. Samples were transported immediately in an icebox to the laboratory for later checks of the baseline salivary pH and *S. mutans* count.

2.4. Oral examination

Oral examination was performed by one dentist using mirrors and explorers under flashlights on the dental chair. The baseline plaque status was evaluated using the PI described by Silness and Loe (1964). Dental caries was described using the DMFT index for permanent teeth and the def index for primary teeth (Petersen et al., 2013).
2.5. Salivary pH evaluation

The collected saliva samples were warmed at 25 °C for 10 min and vortexed for 30 s. A digital pH meter (AD1000, ADWA Instruments Kft., Szeged, Hungary) was used to evaluate the salivary pH.

2.6. Salivary microbial assessment

From each sample, 100 μl were aseptically added to 0.9 ml of saline in a sterile test tube. After serial dilutions, 100 μl of the dilutions was spread onto mitis salivarius-bacitracin supplemented with sterile potassium tellurite solution 1% (Difco–Lawrence, USA) for the S. mutans count.

The plates were anaerobically incubated at 5% CO2 and 37 °C for 48 hrs. S. mutans colonies were identified based on their morphological and biochemical characteristics (Lobo et al., 2014). S. mutans counts in colony-forming units (CFU/ml) were converted to log10 CFU/ml in saliva.

2.7. Test materials

The probiotic lozenges (BioGaia prodentis) contain Lactobacillus reuteri DSM 17,938 and Lactobacillus reuteri ATCC PTA 5289 (Pharma Partners Ltd, 8 Delta Park, Alton, Hampshire, U.K.) manufactured in E.U. under the licensed BioGaia AB, Stockholm, Sweden. CHX gluconate mouthwash (Clorasept) composed of Chlorhexidine Gluconate 0.05% w/v was purchased from SPI-MACO ADDWAEIH, KSA.

2.8. Study design

For one month, participants in the probiotic group consumed one probiotic lozenge daily in the evening after brushing their teeth. Whereas, participants in the CHX group were instructed to rinse with 10 ml of CHX mouthwash diluted in 10 ml of water for 30 s, twice daily (after breakfast and before bedtime), for one month.

No brushing their teeth after consuming the probiotic lozenge and using the CHX mouthwash was allowed for at least one hour. Parents were instructed to immediately notify the investigators in case of any unfavorable reaction.

The control group did not receive any specific instructions; rather they followed the regular oral hygiene measures, including properly brushing their teeth twice daily.

Parents were provided with a printed calendar. Daily marks on the printed calendar were evaluated to check the consumption of the tested materials and the frequency of brushing.

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**Fig. 1** Flow of subjects through the study.
teeth. Moreover, the participants were instructed to return the probiotic lozenge strips and the CHX bottle to check for their compliance.

At the recall visits on the 15th and 30th days, all the above-mentioned parameters (PI, salivary pH, and \(S. \text{mutans}\) count) were investigated.

### 2.9. Statistical analysis

Data were analyzed by the Statistical Package for Social Sciences (SPSS) software, version 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented with the mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to assess the normal distribution of data. Analysis of variance (ANOVA) and the Tukey test was performed to detect changes in PI scores, salivary pH values, and \(S. \text{mutans}\) count throughout the period of the study. A \(P\)-value ≤ 0.05 indicates a significant difference.

### 3. Results

Based on the inclusion criteria (Fig. 1), only 54 children (13 males and 41 females with an average age of 9.87 ± 1.43) were available for this study.

Regarding the caries indices, the mean def value was 3.67 ± 2.91 while the mean DMFT value was 2.69 ± 2.49.

Our results revealed a significant difference in PI scores between the study groups at the 15th and 30th days (\(p\)-value < 0.05) (Table 1). Multiple comparisons between every two groups showed non-significant differences between the probiotic and CHX groups (Table 4).

The salivary pH values on the 30th day showed a significant difference between the studied groups (Table 2). Multiple comparisons between every two groups showed a significant difference on the 30th day between control and both probiotic and CHX groups (Table 4).

Regarding the \(S. \text{mutans}\) count on the 15th and 30th days, significant differences were reported between the study groups (Table 3). Intergroup comparisons revealed significant differences between the control and both probiotic and CHX groups (Table 4).

### 4. Discussion

The results of the present study revealed a significant decrease in PI scores on the 15th and 30th days among both probiotic and CHX groups. These results were in line with Shah et al. (2019), who concluded that probiotics have a similar effect of CHX on plaque control. The anti-plaque effect of \(L. \text{reuteri}\) strains in the probiotic lozenges may be attributed to their ability to prevent the microorganisms from adhesion and growth on the tooth surface, reduce the cytotoxic products by modifying the plaque biochemistry, and inhibit intercellular plaque matrix formation (Raff and Hunt, 2012; Stamatova and Meurman, 2009). Whereas, CHX inhibits the growth of dental plaque through an immediate bactericidal action during its
application and a bacteriostatic action because of its adsorption to the biofilm on the tooth surface (Eden, 2017; Mishra et al., 2019; Shah et al., 2019; Gızligöz et al., 2020).

In the present study, PI scores showed a non-significant difference between probiotic and CHX groups on both the 15th and 30th days, which is inconsistent with the results of Sharma et al. (2019), who found a significant difference in PI scores among the probiotic and CHX groups on the 14th day. This inconsistency could be attributed to the difference in the research design and the microbial strains in the probiotics used in their study.

In the current study, the mean salivary pH values showed a significant increase on the 30th day in both probiotic and CHX groups, with no significant difference between them. These results are in consonance with Srivastava et al. (2016), who reported a significant elevation in salivary pH values after the consumption of probiotic curd. The increase in salivary pH values with probiotic lozenges could be attributed to the arginolytic nature of the probiotics used in their study.

In the present study, probiotic lozenges significantly reduced the mean S. mutans count compared to the control group on the 15th and 30th days. These results are concomitant with Caglar et al. (2008) and Alamoudi et al. (2018), who reported that probiotic lozenges significantly reduce the S. mutans count.

In our study, the least mean S. mutans count recorded in the CHX group and the probiotic group were significantly different. On the contrary, Jothika et al. (2015) reported a non-significant difference between the probiotic and CHX mouthwashes in reducing the S. mutans count.

The L. reuteri strain contained in the probiotic lozenges produces the reuterin compound, which in turn produces oxidative stress in different pathogenic microorganisms (Schaefer et al., 2010; Tang and Lu, 2019), which accounts for its antimicrobial effect against S. mutans. Moreover, reuterin prevents bacterial adherence and colonization (Kang et al., 2011). Moreover, the antimicrobial effects of probiotics may be attributed to other antimicrobial substances comprising organic acids, hydrogen peroxide, bacteriolytic enzymes, and bacteriocins (Wannun et al., 2016; Seminario-Amez et al., 2017). The probiotic lozenges contain two strains, L. reuteri DSM 17938 and L. reuteri ATCC PTA 5289. This combination was proved to prevent co-aggregation and the growth of S. mutans in vitro (Hasslöf et al., 2010).

In the CHX group, a significant reduction in the S. mutans count was recorded on the 15th day. Sharma et al. (2018) reported similar results with the same amount of the CHX mouthwash. On the 30th day, our results revealed a highly significant reduction in the mean S. mutans count in the CHX group, which is inconsistent with the results of the study conducted by Jothika et al. (2015), who reported an increase in the S. mutans count on the 30th day compared to the baseline.

The control group exhibited a significant reduction in PI and S. mutans counts from the baseline to the 30th day. This finding could be due to the consistency brushing of teeth and parental supervision.

**Table 4**  Intergroup comparisons of plaque index scores, salivary pH values, and *Streptococcus mutans* counts (log_{10} CFU/ml).

| Groups   | Plaque index score | Salivary pH values | Streptococcus mutans count (log_{10} CFU/ml) |
|----------|-------------------|--------------------|---------------------------------------------|
|          | n  | Mean ± SD | p value  | n  | Mean ± SD | p value  | n  | Mean ± SD | p-value |
| Baseline |    |           |          |    |           |          |    |           |         |
| Control  | 18 | 1.94 ± 0.73 | 0.802    | 18 | 5.93 ± 0.63 | 0.710    | 18 | 6.79 ± 0.09 | 0.813    |
| Probiotic|    |            |          |    |           |          |    |           |         |
| Control  | 18 | 1.78 ± 0.81 | 0.676    | 18 | 5.93 ± 0.63 | 0.996    | 18 | 6.79 ± 0.09 | 0.869    |
| Chlorhexidine | 18 | 1.72 ± 0.83 | 0.976    | 18 | 5.78 ± 0.44 | 0.763    | 18 | 6.77 ± 0.11 | 0.994    |
| Probiotic|    |            |          |    |           |          |    |           |         |
| Chlorhexidine | 18 | 1.72 ± 0.83 | 0.55     | 18 | 5.91 ± 0.55 | 0.55     | 18 | 6.77 ± 0.08 | 0.55     |
| 15 days  |    |           |          |    |           |          |    |           |         |
| Control  | 18 | 1.11 ± 0.58 | 0.206    | 18 | 6.97 ± 0.17 | 0.877    | 18 | 6.51 ± 0.14 | < 0.001*  |
| Probiotic|    |            |          |    |           |          |    |           |         |
| Control  | 18 | 0.78 ± 0.55 | 0.033*   | 18 | 6.97 ± 0.17 | 0.250    | 18 | 6.51 ± 0.14 | < 0.001*  |
| Chlorhexidine | 18 | 0.61 ± 0.61 | 0.667    | 18 | 7.02 ± 0.23 | 0.504    | 18 | 5.44 ± 0.11 | 0.009*    |
| Probiotic|    |            |          |    |           |          |    |           |         |
| Chlorhexidine | 18 | 0.61 ± 0.61 | 0.52     | 18 | 7.15 ± 0.52 | 0.540    | 18 | 5.31 ± 0.13 | 0.009*    |
| 30 days  |    |           |          |    |           |          |    |           |         |
| Control  | 18 | 1.06 ± 0.54 | < 0.001* | 18 | 6.99 ± 0.16 | 0.009*   | 18 | 5.99 ± 0.39 | < 0.001*  |
| Probiotic|    |            |          |    |           |          |    |           |         |
| Control  | 18 | 0.28 ± 0.46 | < 0.001* | 18 | 7.17 ± 0.15 | 0.003*   | 18 | 5.99 ± 0.39 | < 0.001*  |
| Chlorhexidine | 18 | 0.18 ± 0.39 | 0.800    | 18 | 7.17 ± 0.15 | 0.927    | 18 | 5.40 ± 0.10 | 0.014*    |
| 30 days  |    |           |          |    |           |          |    |           |         |

* Indicates significant differences (p < 0.05).
Children in our study exhibited more acceptance and compliance with probiotic lozenges due to their favorable taste and odor compared to the CHX mouthwash.

The foremost limitations of this study were the small sample size and the short-term follow-up period. Moreover, the study was confined to S. mutans. Therefore, further researches of a longer follow-up period and larger sample size, including different species of cariogenic microorganisms, are needed to assess the effect of long-term use, frequency, and doses of probiotic lozenges on oral health.

5. Conclusion

- Probiotic lozenges and CHX mouthwash significantly reduce plaque accumulation and the S. mutans count and increase the salivary pH values.
- Probiotic lozenges could be an alternative to CHX mouthwash and should be encouraged as an adjunct to brushing teeth and other oral hygiene practices in the control of dental caries.

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CRediT authorship contribution statement

Sara Matuq Badri: Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. Emtenan Hesham Felemban: Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. Ghaida Kamel Alnajjar: Validation, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Funding acquisition. Fadwa Monawar Aloitaib: Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. Shoroq Talin Aljahdali: Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. Yahia Ahmed Maher: Conceptualization, Methodology, Visualization, Supervision, Project administration, Visualization. Adel Fathi: Conceptualization, Methodology, Validation, Resources, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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