Effectiveness of Oral Probiotics in Reducing *S. Mutans* Count in Caries-active Children: A Comparison with Chlorhexidine and Herbal Mouthrinse (Hiora®)

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**ABSTRACT**

**Aim:** The purpose of this study was to compare the reduction in salivary mutans streptococci counts and to evaluate any change in the salivary pH values after the use of oral probiotics, chlorhexidine, and herbal mouthrinse.

**Method:** Oral probiotics, chlorhexidine, and herbal mouthrinse (Hiora®) were evaluated for their efficacy against *S. mutans* in 75 caries-active children, aged 6–14 years.

**Results:** The values obtained were subjected to statistical analysis. Oral probiotics showed lesser reduction in the *S. mutans* count as compared to chlorhexidine but the values were statistically insignificant. Herbal mouthrinse was not as effective as the oral probiotics or chlorhexidine in reducing the *S. mutans* count.

**Conclusion:** Oral probiotics show a similar efficacy as the gold standard, chlorhexidine, in reduction of oral *S. mutans*.

**Keywords:** Dental caries, Mouthrinses, Probiotics, *S. mutans*.

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**INTRODUCTION**

Dental caries is a result of the interplay of several complex factors, the management of which is a challenge in modern-day dentistry. This has led to an immense suffering and economic burden among different strata of population. Therefore, there has been a paradigm shift from the old restorative strategies for treating caries to newer strategies which emphasize on disease prevention and conservation of tooth structure.

Over the last few decades, various strategies have been used for caries prevention like professional fluoride application, pit & fissure sealants, remineralizing agents, etc. There is another simpler method of dispensing agents for caries prevention which is by the use of mouthrinses. These are readily available, easy for small children to use and cost effective vehicles that can be used as a part of daily oral care regime.

Chlorhexidine mouthrinse has been the gold standard in chemotherapeutic management of plaque control and it is found to be the most effective against all strains of *Streptococcus mutans*.1 It is a cationic bis-biguanide and belongs to the broad spectrum antiseptic group, which acts against gram-positive, gram-negative bacteria, and fungi. It kills the microorganisms by disrupting their cell membrane. The major advantage of chlorhexidine over most other compounds lies in its substantivity, although it is not free from limitations such as brown staining of teeth, altered taste sensation, dry mouth after prolonged use, and has an unpleasant taste. These drawbacks have led researchers to inspect the possible use of plants extracts and natural medicinal herbs as antimicrobial agents.2

Derivatives of plants and their extracts are now being used as remedies to treat disorders and to maintain good health. Herbal mouthwashes are beneficial as they comprise of natural ingredients called phytochemicals.3 One such herbal mouthrinse is Hiora® [Himalaya Herbals] that contains Pilu [Salvadora persica], Nagavalli [Piper betle], Bibhitaki [Terminalia bellerica], Gandhapura taila [Gaultheria fragrantissima], Ela [Elettaria cardamomum], Peppermint satva [Mentha piperita], and Yavani satva [Trachyspermum ammi]. They possess the desired antimicrobial, anti-inflammatory effects, and anticarcinogenic properties.4

One of the emerging approach which is currently being researched for caries prevention is probiotics. The concept of probiotics was given by Dr Metchnikoff in 1908. Lilley and Stillwell introduced the term probiotics, as an antonym to the term “antibiotics.” After its successful results in the human gut, probiotics have been exclusively introduced for the oral cavity. Oral probiotics are live micro-organisms which, when administered in adequate amounts improves the micro-ecological
Oral Probiotics for Reducing Dental Caries in Children

It is stated that probiotics have the capacity to compete, antagonize, and prevent the proliferation of cariogenic bacteria, thus, preventing dental caries. Oral probiotics, ProBiora Plus [Oragenics Inc., Alachua, FL, USA] formerly known as Evora Plus. It comprises a proprietary blend of three select species [ProBiora3] of Streptococcus namely S. oralis KJ3sm, S. uberis KJ2sm, and S. rattus JH145. It contains tablets for 30 day supply and at least 10^{8} CFU of each strain per tablet is present.

Oral probiotics are commercially available in the form of tablets, lozenges, freeze-dried powder, straws, ice-creams, etc.

There are only a few studies reported that compare the effectiveness of oral rinses in reducing the S. mutans count in the oral cavity. Hence, the aim of the present study was to compare the efficacy of oral probiotics with chlorhexidine and Hiora in reducing salivary S. mutans count in caries active children.

Methods

Study Design

The study was conducted in the Department of Pedodontics and Preventive Dentistry. The project was ethically approved by the Institutional Ethical Committee [IDST/IERBC/2017-20] and followed the Consort guidelines. The investigation employed a randomized clinical design with the total duration of 21 days.

The power of the study was seen to be 80% with a sample comprising of 75 children [aged 6–14 years]. Healthy children presenting with one or more interproximal carious lesions, free from any other pathologic condition of teeth and surrounding tissues, with no history of antibiotic use in the past 1 month were included in this trial.

Clinical Part

The participants were clinically examined by a single examiner, with the help of a mouth mirror and an explorer for one or more interproximal carious lesions. The study procedure was explained to the parents or guardian and a written informed consent was obtained from them for participation in the study. A total of 417 children were examined out of which 75 children fulfilled the inclusion criteria.

Fig. 1: Showing materials used in the study

Division of Groups

Group A [n = 25]: Oral probiotic tablet [EvoraPlus^TM^]
Group B [n = 25]: 0.2% chlorhexidine digluconate [Hexidine^®^]
Group C [n = 25]: herbal oral rinse [Himalaya Herbals Hiora^®^]

All the mouthwashes were prescribed to be used twice daily for full 1 minute in the interval of 12 hours for 14 days.

In the study, the designated rinse was dispensed by the investigator to each subject in a sealed bottle without knowing the identity of the rinse. The allocation of the participants was based on SNOSE concealment. The patients were assigned a piece of paper comprising of a randomized group number sealed in the dark colored envelope containing respective serial number over it. The envelope was opened once the intervention was assigned. Based on the group assigned in the paper, sealed mouthrinses were handed over to the subjects [Fig. 1].

On the day of sample collection, all the children were instructed to have an early morning breakfast in order to maintain a specified 90 minutes gap before collecting samples. This time gap was ensured to avoid any influence of food consumption on the salivary composition. Saliva sampling was performed between 9 and 10 a.m. in the morning to minimize the effect of diurnal variation. The child was made to chew on a paraffin block for 2 minutes sitting in an erect position on the dental chair. Approximately 2 to 3 ml of the stimulated saliva was collected in properly labeled sterile containers [Fig. 2].

The salivary sample was examined for pH immediately after collection with a pH meter [Fig. 3] and then stored at 4°C till microbiological evaluation for S. mutans count. The pH apparatus was cleansed with water and dried after every use. Saliva samples were collected from each participant at three intervals. The first sample was collected at baseline, after which the children were given their designated mouthrinses. The parents were requested to assist their children in the rinsing protocol twice daily half an hour after brushing in the morning and at night for a period of 2 weeks that is, 14 days. The children were asked to maintain normal oral hygiene protocol apart from the mouthrinse.

The next salivary sample was collected on the 14th day since the beginning of the intervention.

The children were made to discontinue the rinse after 14 days and the last saliva sample was collected after 1 week of discontinuation that is, 21st day from baseline to evaluate the sustenance of reduction in microbial counts, if any.

Fig. 2: Showing saliva sample collection
Laboratory Part

Mitis-Salivarius-Bacitracin agar [Gold et al.] was used as the media for S. mutans culture. The plates were incubated within 4 hours at 37°C for 48 hours under 5 to 20% CO₂. Streptococcus mutans colonies were identified as highly convex, round or spherical, raised, dark blue in color, ranging from a pinpoint to pin head size with a rough surface using digital colony counter [Fig. 4]. The results were expressed in CFU. All plates were processed and examined by the same investigator. Confirmation of mutans streptococci was performed under light microscope.⁸

Statistical Analysis

The Excel and SPSS [SPSS Inc, Chicago, Version 21.] software packages were used for data entry and analysis. Mean values, ANOVA, and post hoc Bonferroni test was used for evaluating the data.

RESULTS

The mean DMFT values of participants in groups A, B, and C were 2.20 ± 0.941, 2.40 ± 1.549, and 2.46 ± 1.457, respectively and these were found to be statistically nonsignificant. The mean CFU values of the three groups at baseline were 2.99 ± 1.36, 4.50 ± 3.89, and 7.17 ± 0.31, respectively. These were statistically nonsignificant.

Evaluation of CFU after 14 Days

All three groups exhibited marked reduction in S. mutans counts over a period of 14 days. The mean CFU in the Oral probiotics group after 14 days was 1.03 ± 0.91* and in the Chlorhexidine groups it was 0.70 ± 0.66*. Hiora showed a mean CFU of 2.71 ± 2.04* after 14 days. It can be seen from Table 1 that mean change in CFU was maximum in Chlorhexidine group and minimum in Hiora. Group A and B showed a comparable reduction. The reduction in all the groups was statistically significant with p value of 0.0001 [Table 2].

Evaluation of CFU after 21 Days

The mean CFU at 21 days after intervention was 0.34 ± 0.46*, 0.19 ± 0.45*, and 2.86 ± 1.80* for groups A, B, and C, respectively. A reduction in CFU in all the three groups was seen after discontinuation of the mouthrinse. Maximum reduction was in group Chlorhexidine and minimum in group Hiora. The values were statistically significant.

Analysis of pH in All 3 Groups

On estimating the baseline pH, the values in all three groups did not differ significantly. They were 7.27 ± 0.31, 7.05 ± 0.28, and 7.17 ± 0.31, respectively.

However, there was a statistically significant difference in the mean pH on 14th day in all the groups. The mean pH values on 14th day of group A, B, and C were 7.06 ± 0.14*, 7.29 ± 0.19*, and 6.96 ± 0.18*, respectively. While group A and C showed a marginal reduction of pH, group B showed a rise in pH. The p value was 0.0001.

The mean pH on 21st day showed no statistical significant difference in the values. The mean pH values of the three groups were 7.07 ± 0.14, 7.18 ± 0.12, and 7.00 ± 0.37, respectively.

Table 1: Table showing mean change in CFU and pH values of all groups at baseline, 14th day and 21st day

| Groups | Baseline–14th day | 14th day– 21st day | 21st day – baseline |
|--------|------------------|------------------|------------------|
| Group A |                  |                  |                  |
| Mean CFU change | 1.96 ± 0.98* | 0.68 ± 0.60* | 2.64 ± 1.22* |
| Mean pH change | 0.21 ± 0.31 | 0.0067 ± 0.15 | 0.21 ± 0.34* |
| Group B |                  |                  |                  |
| Mean CFU change | 3.80 ± 3.78* | 0.50 ± 0.80* | 4.31 ± 3.52* |
| Mean pH change | -0.24 ± 0.26* | 0.11 ± 0.21 | -0.12 ± 0.35 |
| Group C |                  |                  |                  |
| Mean CFU change | 1.02 ± 1.89 | -0.15 ± 1.56 | 0.86 ± 1.88 |
| Mean pH change | 0.21 ± 0.35* | 0.04 ± 0.39 | 0.17 ± 0.51 |

CFU, colony-forming units (*Significance)
Group 3 showed rise in pH after discontinuing the oral rinse. The p value was 0.134 [Table 1].

**Discussion**

Streptococcus mutans has been implicated as one of the virulent factors for causing caries. 

It has been seen to possess the peculiar ability to adhere to salivary pellicle and play an important role in initiation of caries. Alaluusua and Renkonen studied Streptococcus mutans colonization in plaque and saliva from 39 children aged 2–4 years from the suburbs of Helsinki and confirmed its close relationship to dental caries. 

Rogers isolated 82 streptococcal strains from the mouth of individuals aged 13–25 years with active caries and found two biotypes to be related to the species *S. sanguis* and *S. mutans*. The present study considered assessing the reduction in levels of Streptococcus mutans with the use of different mouthrinses.

Salivary levels of *S. mutans* are shown to be directly related to the number of tooth sites colonized and to their proportion in dental plaque. It has been stated that there is positive correlation concentration of mutans streptococci in saliva and dental caries.

Pannu et al. reported that individuals with lower concentrations of mutans streptococci in their saliva showed a significantly lower mean number of decayed surfaces.

The use of mouthrinses is a common practice in oral care routine of populations worldwide. Hence, it would be desirable to incorporate therapeutic agents in mouthrinses for caries prevention. In the present study, three mouthrinses, Prodiora Plus, chlorhexidine, and Hiora were evaluated for their effectiveness in reducing the *S. mutans* count [Fig. 1]. The results of the present study revealed that chlorhexidine had the highest antimicrobial efficacy against *S. mutans* out of the three, though the efficacy of probiotic was similar to chlorhexidine. Hiora was least effective [Table 1].

Chlorhexidine has been studied extensively and seen to reduce the total salivary number of *S. mutans* to nondetectable levels by Kristoffersson and Bratthall.

Maltz et al. reported reduction in the salivary *S. mutans* counts by chlorhexidine in school children with 6 months follow up.

Lijjemark et al. observed that chlorhexidine blocked adherence of *S. mutans* to dextran-coated hydroxyapatite.

Järvinen et al. showed that CHX was highly effective against all the *S. mutans* isolates. A herbal mouthrinse was compared with chlorhexidine by Nagappan N and John J and found chlorhexidine to be more effective against streptococcus mutans. These findings were in accordance with the present study.

Subramaniam F, Gupta T evaluated the efficacy of Hiora on oral microbial load in Down syndrome children when compared to chlorhexidine. They found better results with chlorhexidine (Oragard).

A similar study was conducted by Mishra R et al. in which the effect of chlorhexidine, Evora plus (probiotic) and a herbal mouthrinse was studied on C. albicans in children. They found chlorhexidine to be most effective and herbal rinse to be the least whereas Evora was comparable to chlorhexidine. Sharma et al. observed that Hiora was least effective when compared to chlorhexidine and sodium fluoride mouthrinse against *S. mutans* after using for 15 days.

On the contrary, Ramamurthy J compared the efficacy of chlorhexidine and Hiora mouth wash gingivitis patients for 15 days and found both mouthwashes to be equally effective in treating gingivitis. Jaidka S et al. found that Hiora showed maximum reduction in plaque accumulation, gingival inflammation, and microbial load followed by xylitol and chlorhexidine after 30 days in caries free children.

Gupta R et al. stated that Hiora and T. chebula could be used as alternative mouthwashes to chlorhexidine as they reported no side effects in children.

Prodiora Plus, in the present study, was seen to be comparable with chlorhexidine, in its effect against *S. mutans*, though the mechanism of action of both is completely different. *S. oralis* (previously called *S. sanguis* type II) and *S. uberis* are known to produce hydrogen peroxide which inhibits the growth of certain pathogens [Hillman and Socransky; Hillman et al., Hillman and Shivers]. S. rattus is the lactic acid-deficient strain which is a very close relative of *S. mutans*. It was once classified as *S. mutans* strain [Coykendall 1989] and thus should be able to compete for the same binding sites on the tooth surface, nutrients, and other resources with the indigenous, decay-causing *S. mutans*. Since it does not produce lactic acid, it is virtually incapable of causing dental caries [Johnston et al.]. This specific strain, S. rattus JH145, selected for maintaining dental health most likely uses a competitive exclusion mechanism to reduce the numbers of *S. mutans* cells present on teeth.

In 2009, Zahradik RT et al. found that Prodiora3 substantially reduced the levels of dental pathogens including *S. mutans* in saliva and gingiva. They stated that Prodiora3 is safe to use and helps to maintain a healthy oral bacterial composition. There is literature that supports the fact that these probiotic tablets reduce the levels of mutans streptococci in saliva and plaque.

The strains of Prodiora3 are expected to reach and saturate their target habitats within few weeks of regular use. This can be appreciated in the present study as even after discontinuation of the rinse, a drop in the levels of *S. mutans* could be found after 1 week. Twetman and Stecksen-Blicks stated that the sooner the probiotics colonize the oral cavity, the long term effects will be better, especially when the probiotics are consumed daily. They also observed that the probiotic strains can be detected up to 10 to 12 days after last ingestion.

Strus et al. and Roberfroid suggested that the probiotic bacteria protect the oral health by competing with oral pathogens for nutrients, growth factors, and adhesion sites. Once these bacteria adhere into the oral cavity, they aggregate and inhibit the adhesion of harmful micro-organisms by producing bacteriocins, antimicrobial compounds such as acids or peroxides and adhesion inhibitors.

Hedayati-Hajikand et al. evaluated the effect of probiotic chewing tablets on early childhood caries in 2–3 years old children. They suggested that early childhood caries could be reduced by using these tablets [Prodiora] as an adjunct to daily use of fluoride toothpaste in preschool children.

Elahi et al. and Stamatova et al. observed significant reduction in C. albicans counts after oral administration of probiotics and they stated that oral probiotics

| Group (I) | Group (J) | Mean difference (14th day) | p-value | Mean difference (21st day) | p-value |
|----------|-----------|-----------------------------|---------|-----------------------------|---------|
| A        | B         | 0.32                        | 1.00    | 0.14                        | 1.00    |
|          | C         | -1.68                       | 0.004   | -2.52                       | 0.0001  |
| B        | C         | -2.00                       | 0.001   | -2.67                       | 0.0001  |

Post hoc bonferroni applied, *significance at p < 0.05
are proven to be effective in reducing specific micro-organisms such as Candida and Streptococci spp.24,25

The present study results are in favor of using oral probiotics for children and suggest that the oral administration of probiotics may help maintain and decrease the low counts of cariogenic pathogens. Another parameter of the present study was evaluation of pH during the intervention. The mean pH of all the subjects at baseline was found to be close to neutral and comparable. After 14 days ProBiora Plus showed a marginal fall in the pH \( p = 0.0001 \) whereas the other two groups showed a slight increase in pH \( p = 0.0001 \), although all the values were close to neutral (Table 1).

**CONCLUSION**

Although the mechanism of action of probiotics is yet unexplained, as compared to chlorhexidine, which is an antimicrobial, the probiotics replenish the oral cavity with health promoting strains of S. mutans which could be more desirable for long term health benefits. However, studies with a larger sample size are required to be undertaken to understand the definitive therapeutic actions of oral probiotic as an oral rinse.

**CLINICAL SIGNIFICANCE**

This study stresses on the beneficial effects of oral probiotics for the management of dental caries and maintenance of oral health in children.

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