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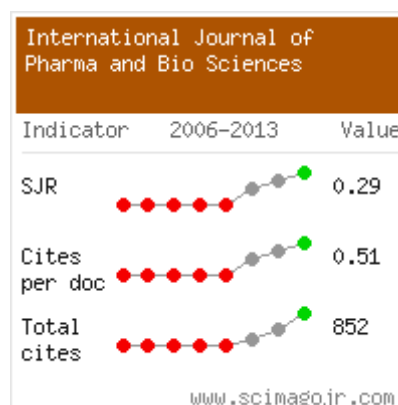
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**PROBIOTICS IN PERIODONTAL THERAPY****LITTY SCARIYA<sup>\*1</sup>, NAGARATHNA D.V<sup>2</sup> AND MERLINE VARGHESE<sup>1</sup>**

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**ABSTRACT**

To partially assess the efficacy of probiotic lozenges, in the treatment of periodontal disease. Material and methods: Twenty eight subjects, of both sexes, were selected and divided into 4 groups (2 test groups and 2 control groups). The test group was instructed to consume probiotic lozenges whereas the control group did not receive any probiotic product. Clinical parameters such as plaque index, gingival index, modified sulcular bleeding index and probing pocket depth were recorded and assessed at baseline, day 15, 30, 45 and day 60. The Test group showed significant reduction in all parameters when compared to that of Control group. After stopping probiotic administration on day 30, the test group showed a significant increase in all the clinical parameters except probing pocket depth on day 45 and day 60. Conclusions: The results show that probiotic lozenges were efficacious in reducing both moderate to severe gingivitis and moderate periodontitis.

**KEY WORDS:** Probiotics, Gingivitis, Periodontitis

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## INTRODUCTION

The World Health Organization defines probiotics as "living organisms, principally bacteria that are safe for human consumption and when ingested in sufficient quantities, have beneficial effects on human health, beyond the basic nutrition"<sup>1</sup>. The concept of probiotics dates back to the 20<sup>th</sup> century when Ukrainian bacteriologist and Nobel laureate, Elie Metchnikoff laid down the scientific foundation of probiotic. He proposed that Bulgarian people had longer longevity due to fermented milk containing viable bacteria. The term 'probiotics', the antonym for the term antibiotics, was introduced in 1965 by Lilly and Stillwell as substances produced by microorganisms which promote the growth of other microorganisms<sup>1</sup>. Probiotic therapy has been studied extensively in a variety of systemic indications and medical disorders and have also been introduced in the field of periodontal healthcare. The discovery of the role of free radicals in cancer, diabetes, cardiovascular diseases, and other chronic diseases, including periodontal disease has led to the emergence of antioxidants as prophylactic and therapeutic agents<sup>2</sup>. The development of resistance to antibiotics has raised the possibility of a return to the pre-antibiotic dark ages. Here, probiotics provide an effective alternative way, which is economical and natural to combat periodontal disease<sup>3</sup>. The aim of this study was to evaluate the efficacy of orally administered probiotic lozenges in the treatment of chronic gingival and periodontal disease by evaluating changes in monitored clinical parameters. Lozenges containing *Streptococcus salivarius* were selected because of their innate capacity to bind and persist on the tongue dorsum. Some strains of *Streptococcus salivarius* release into saliva, copious quantities of bacteriocins that could provide a targeted way of removing deleterious bacteria making them a more effective probiotic organism. They also regularly produce the enzymes dextranase and urease, which could help reduce dental plaque accumulation and acidification, respectively<sup>4</sup>.

## MATERIALS AND METHODS

### (i) Materials used

Lozenges containing not less than 100 million *Streptococcus salivarius* bacteria per tablet were imported from BLIS Technologies Ltd, Dunedin New Zealand.

### (ii) Methods of Randomisation of Subjects

This study was conducted on 28 subjects between the age of 20 and 60 years of age. Subjects were selected from those attending the Department of Periodontics, A.J Dental College and Hospital, Mangalore. Subjects were selected on the basis of the following criteria by examining the periodontium.

#### Inclusion criteria

1. Good general health and age ranges between 20 to 60years
2. Not participated in any clinical trial during the previous 4 weeks
3. No ongoing antibiotic treatment
4. Only individuals with moderate and severe gingivitis, and moderate periodontitis

#### Exclusion criteria

1. Individuals with systemic disease predisposing to periodontitis.
2. Individuals with probing pocket depth more than 6mm.
3. Presence of tooth with grade II or grade III mobility or abscess formation.
4. Pregnancy or breastfeeding
5. Physical or mental handicaps that may interfere with an adequate oral hygiene.
6. History of drug abuse
7. Allergies

#### Groups

The selected subjects were divided into four groups, with 7 subjects in each group.

#### Group1:Test

Seven male subjects with gingival index score 3 or 2 with periodontal pocket less than 6 mm treated with probiotic lozenges.

#### Group2:Test

Seven female subjects with gingival index

scores 3 or 2 with periodontal pocket less than 6 mm treated with probiotic lozenges.

### **Group3:Control**

Seven male subjects with gingival index scores 3 or 2 with periodontal pocket less than 6mm treated without probiotic lozenges.

### **Group4:Control**

Seven female subjects with gingival index scores 3 or 2 with periodontal pocket less than 6mm treated without probiotic lozenges. The participants were briefed in detail regarding the study. The proposed study was reviewed by the ethical committee of the institution and clearance was obtained. An informed consent was obtained from each subject before conducting the trial. Preselected participants were scheduled for a dental examination. The subjects were allotted into groups by a second post graduate student, while the clinician conducting the clinical examinations was not informed whether subjects were actively taking the lozenges or not. The test group subjects were instructed to store the lozenges in a refrigerator, as recommended by the manufacturer.

### **Study protocol**

The study period was 60 days. Subjects in Group1 and Group2, after initial scaling and root planning, were instructed to consume 2 lozenges containing *Streptococcus salivarius* M18 every day for the next 30 days. Subjects in Group 3 and Group 4 were not instructed to consume any lozenges but underwent scaling and root planning.

Participants in Group1 and Group2 were directed to place one lozenge in their oral cavity for few minutes after brushing their teeth once in the morning and in the evening, allowing the tablet to dissolve.

The patients were also instructed on how to brush and floss effectively. Participants in the test group were instructed to bring the remaining lozenges during their visits to the hospital. A count of the remaining lozenges was taken to monitor whether the subjects were regularly consuming the lozenges. Clinical parameters were obtained for all the subjects on day 0 (Baseline); day 15, day 30, day 45, and day 60. All 7 subjects in each group were analysed. At the end of the study period, 6 M18 tablets were returned to BLIS Technologies Ltd New Zealand for quality assurance testing.

## **RESULTS**

The following clinical parameters were assessed in all subjects during each visit.

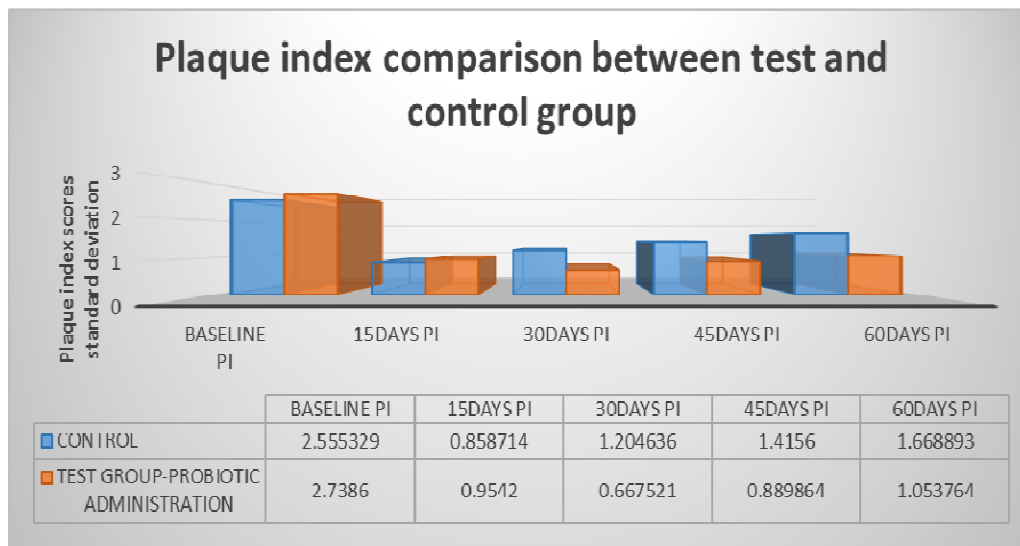
1. Supragingival plaque was scored by Plaque Index (P.I) (Silness and Loe1964).
2. The Gingival Index (G.I) (Loe and Silness 1963) was scored.
3. Bleeding on probing by The Modified Sulcular Bleeding Index (mSBI) by Mombelli et al 1987.
4. Probing pocket depth (PPD) measured using Williams Periodontal Probe.

### **Data Analysis**

The data was tabulated in Microsoft excel and analysed using SPSS (Statistical Product and Service Solutions)version-16.

The comparison between test and control group in each category (PI-plaque index, GI-gingival index, mSBI-modified sulcular bleeding index, PD-probing pocket depth) at each interval was done using an independent T test. The level of significance was set to  $p < 0.05$  (where 'p' is the probability value).

**Plaque Index Comparison Between Test And Control Group**

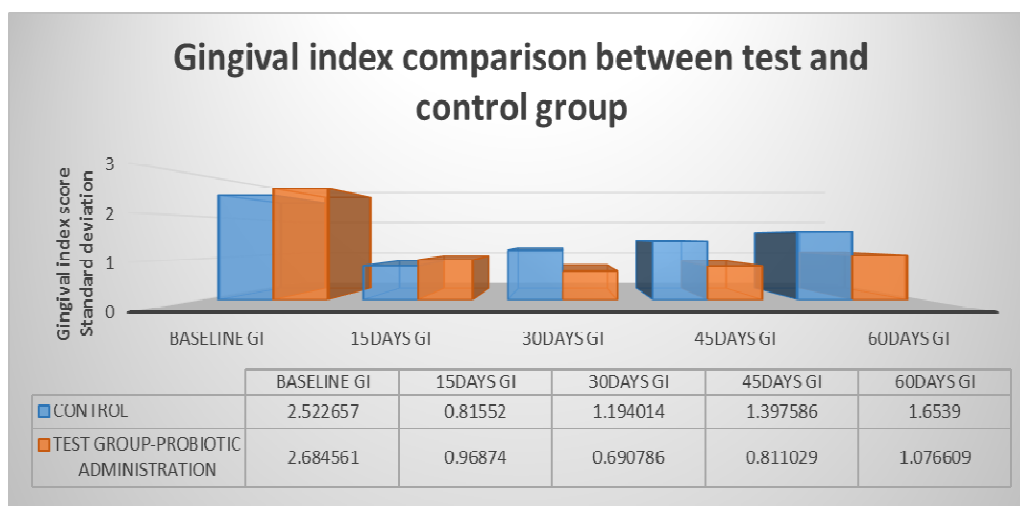


**Graph 1**  
**Plaque index Comparison between Control and Test Group**

Graph 1 shows the plaque index (PI) scores for both test and control group at baseline, day 15, day 30 day, 45 and day 60. The mean score in Test and Control groups were almost similar at base line. On day 15 the mean score was reduced in both groups. At day 30 the mean plaque index score of the Test group was significantly lower when compared to that of the control group for which there was an increase in the score compared to day 15. There was an increase in the mean

score of PI in the test group on day 45 and day 60, whereas in the control group there was a continuous increase in the mean score from day 15 to day 30, day 45 and day 60 respectively. There was no statistically significant difference between the plaque index scores of test and control group at baseline and day 15 ( $p>0.05$ ). The difference between two group on day 30 day 45 and 60 was statistically significant ( $p<0.001$ ).

**Gingival Index Comparison Between Test And Control Group**

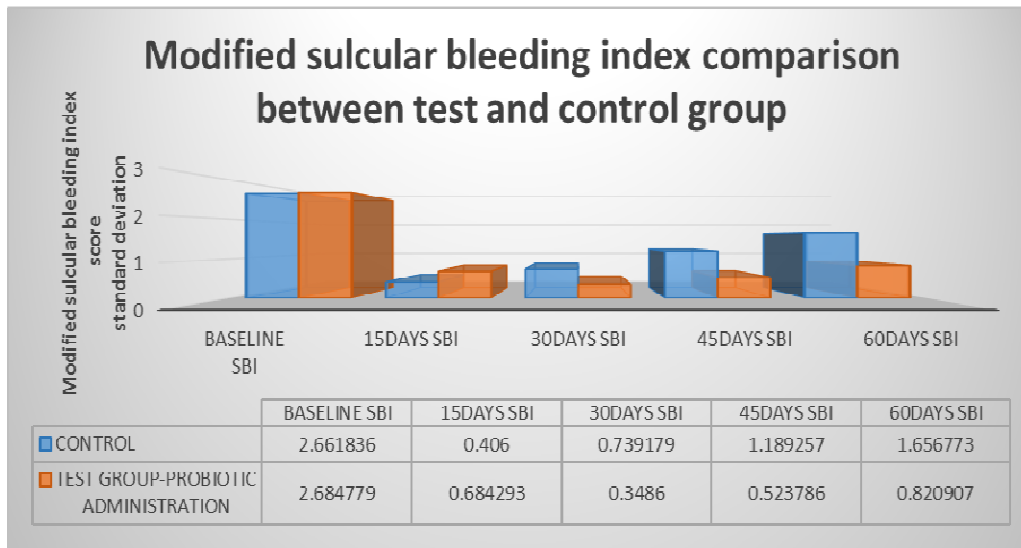


**Graph 2**  
**Gingival Index Comparison between Control and Test Group**

Graph 2 shows the gingival index scores for both test and control groups at baseline, day 15, day 30, day 45 and day 60. There was no statistically significant difference between the two groups at baseline and day 15. The difference between the two groups on day 30, day 45, and day 60 was significantly higher ( $p < 0.001$ ). The mean score of GI in the test and control group at baseline were similar. There was a reduction in the mean score of GI for both groups on day

15. The Test group showed a significant reduction in GI mean score on day 30 when compared to that of the control group which showed an increased GI score when compared to day 15. Similarly to the plaque index results, the mean GI score showed an increase on day 45 and day 60 in the Test group after stopping the administration of probiotic lozenges. The control group showed an increase in mean score from day 15 to day 30, day 45 and day 60 respectively.

**Modified Sulcular Bleeding Index Comparison Between Test And Control Group.**

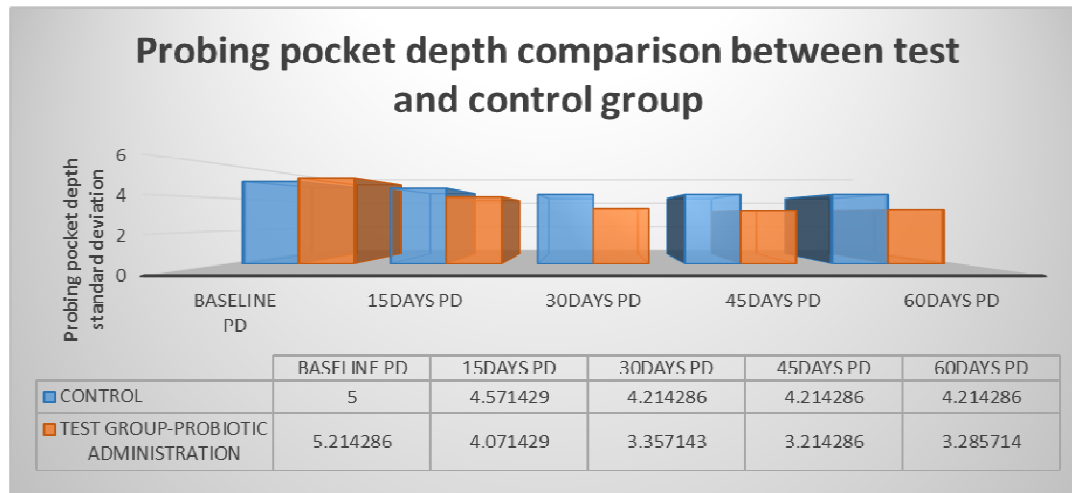


**Graph 3**  
**Modified Sulcular Bleeding Index Comparison between Control Group and Test Group.**

Graph 3 shows the modified sulcular bleeding index (mSBI) scores for both the test and control group at baseline, day 15, day 30, day 45 and day 60. The mean mSBI score for the test group has continued to reduce from baseline to day 30 during the time the tablets were administered after which the mean mSBI scores are observed to increase. Although the control group's mean mSBI score are

seen to decrease on day 15. The mean mSBI score of the control group shows a sharp increase on day 30, day 45 and day 60. There was no statistically significant difference between the two groups at baseline. On day 15 the difference between the 2 groups was significant ( $p = 0.017$ ). The difference between the two groups on day 30, day 45 and day 60 was significantly high ( $p < 0.001$ ).



**Probing Pocket Depth Comparison Between Test And Control****Graph 4****Probing Pocket Depth Comparison Between Test Group and Control Group**

Graph 4 shows the Probing Pocket Depth (PPD) scores for both the test and control group at baseline, day 15, day 30, day 45 and day 60. The mean score PPD at baseline were similar in the Test and the Control groups and on day 15 both group showed a slight reduction in mean score which can be credited to scaling and root planning. On day 30, the Test group showed greater reduction in scores when compared to that of Control group which may be due to probiotic lozenges but on day 45 and day 60 there was no increase or decrease in mean score of both groups. There was no statistically significant difference between the PPD scores of test and control group at baseline and day 15 ( $p > 0.05$ ). The difference between the two groups on day 30, day 45 and day 60 was statistically significant ( $p < 0.05$ ).

**DISCUSSION**

Probiotic bacteria, generally regarded as safe, may favour periodontal health if they are able to establish themselves in oral biofilm and inhibit pathogen growth and metabolism<sup>5</sup>. Various studies on effects of probiotic therapy showed positive results for gastrointestinal disorders as well as for caries associated risk factors<sup>6,7,8,9,10</sup>. However, there are only a few studies which have investigated the influence of probiotics on gingivitis or periodontitis. Twetman et al.

reported a reduction of clinical symptoms caused by gingivitis after the use of chewing gum containing *Lactobacillus reuteri* for two weeks.<sup>11</sup> Krasse et al. documented the effects of probiotic microorganism *Lactobacillus reuteri* for a 2 week period during which gingival inflammation was significantly reduced<sup>12</sup>. It was also demonstrated that probiotic bacteria accumulated in microbial biofilms thus replacing or reducing pathogenic bacteria<sup>13</sup>. Ishikawa et al<sup>14</sup> and Matsuoka et al<sup>15</sup> demonstrated that the use of probiotic pills containing *L.salivarius* significantly reduced the concentration of the periodontopathic bacterium *P.gingivalis* in saliva and subgingival plaque in healthy volunteers. Shimauchi et al<sup>16</sup> documented a reduced concentration of periodontopathic bacteria after administration of probiotic Lactobacilli over a period of weeks, which was associated with improved periodontal conditions<sup>17</sup>. It is well known that the effect of professional cleaning of teeth is effective in short term treatment of gingivitis. In the present study there was no difference in mean scores between the Test and Control group at base line. On day 15 both the groups showed a reduction in PI, GI, mSBI, and PD. This may be because scaling and root planning was carried out in both groups. A patient's ability to maintain oral hygiene may also be a factor. But on day 30, the



test group exhibited a greater reduction in their PI score than did the control group. Similar results were obtained in a study conducted by Shimauchi et al<sup>16</sup>. The test subjects also showed a significant reduction in the GI score when compared to the control group. Similar results were obtained in a study conducted by Krasse et al<sup>12</sup>, Shimauchi et al<sup>16</sup>, Della Riccia et al<sup>18</sup>. In the case of the mSBI, a significant reduction in the score on day 30 was observed in the test group when compared to the control. On day 45 and day 60, the score increased, i.e. the number of bleeding site increased as soon as the probiotic intake was stopped. Similar observations were seen in the study by Twetman et al<sup>11</sup>. On day 30, a significant beneficial effect of the probiotic treatment was observed for the PPD based on comparison with the control group. Similar findings were reported in the study conducted by Matsuoka et al<sup>15</sup>, Shimauchi et al<sup>16</sup>. In general, beneficial effects from a probiotic will only take place as long as the probiotic is applied. Therefore probiotic therapy should not be seen as a treatment that permanently alters the oral microbiota as evidence indicates they are not able to sustain a shift to a stable non-pathogenic microbiota<sup>19</sup>. This observation is supported by the present study with beneficial effects being most obvious during the actual dosing phase for all parameters monitored. The reduction in all clinical parameters in the Test group appear to be due to administration of the probiotic lozenges. Once the administration of probiotic lozenge was stopped all the clinical parameters (PI, GI, mSBI) showed increases in their mean scores although generally they remained lower than the corresponding scores for the control group with the exception of PPD, which almost remained constant. It was not possible to determine why mean PPD scores remained constant after an initial reduction. The mean score of all the clinical parameters (PI, GI, mSBI) except PPD increased in the control group from day

15 to day 30, 45 and 60. PPD remained constant on day 30, day 45 and day 60. Despite the effect of professional cleaning, the reduction in all indexes was stronger and significantly better than the control group in subjects supplemented with probiotics lozenges.

### **QUALITY ASSURANCE TEST**

The test report concludes that the levels of *Streptococcus salivarius* M18 in the tablet may not have been optimal during the entire course of the trial, although it is likely that there should still be enough live probiotic bacteria to impact on the oral health of the test subjects.

### **CONCLUSION**

#### **Limitations of this study are as follows:**

1. Microbial analysis not carried out.
2. All lozenges were not refrigerated.
3. Study was not restricted to any particular social strata.
4. Cell count conducted on sample tablets after the study period found that levels of *S. salivarius* M18 may not have been optimal during the entire course of the trial. Despite some of these limitations, the reduction in all indices monitored was seen to be stronger and significantly better in all subjects that were supplemented with probiotic M18 tablets than in the control group who were not administered with any probiotic. This study concludes that *Streptococcus salivarius* M18 may be potentially useful as an aid in improving the oral health of periodontal patients. Further studies including microbial analysis need to be performed to confirm the initial findings of this report. The effect of probiotics on different strata's of Indian society and the survivability of *Streptococcus salivarius* M18 bacteria in different climatic conditions such as that found in India, also need to be further probed.

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