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ORIGINAL RESEARCH

Efficacy of Oral Probiotics as an Adjunct to Scaling and Root Planing in Nonsurgical Treatment Outcome of Generalized Chronic Periodontitis Patients: A Clinico-Microbiological Study

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ABSTRACT

Aim: This study aims to find out the effects of Probiotics, delivered with a lozenge for its effect on clinical and microbiological parameters in subjects with chronic periodontitis after scaling and root planing (SRP).

Materials and methods: A total of 40 generalized mild to moderate chronic periodontitis subjects were finally enrolled in a double-blind, placebo-controlled, randomized clinical study. Selected subjects after SRP were randomly divided into two groups: Group I (test group) with 20 subjects receiving probiotic tablet once daily and group II (control group) receiving placebo tablets once daily. Clinical parameters and bacterial count for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* were evaluated at baseline, 2, and 4 months after the medication.

Results: On comparative evaluation between the two groups, results indicated that group I (probiotic group) exhibited statistically significant reduction in both clinical and microbiological levels than group II (control group) over the entire span of the study.

Conclusion: Our results proved that daily oral supplementation of probiotics could be a useful adjunct to SRP in chronic periodontitis patients.

Keywords: Hyperbiotics, Periodontitis, Probiotics.

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INTRODUCTION

Mouth acts as a window to a lot of systemic diseases and serves as a port of entry of the various infections that can alter and affect the immune status of the person. The oral cavity has the potential to harbor at least 600 different bacterial species, and in any given patient, more than 150 species may be present, surfaces of tooth can have as many as billion bacteria in its attached bacterial plaque and good oral hygiene is the fundamental for oral integrity as it greatly affects the quality of life.[1] Periodontitis is a destructive inflammatory disease of the supporting tissues of the teeth and is caused by specific microorganisms or group of specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession, or both. The host responds to the periodontal infections with an array of events involving both innate and adaptive immunity.[2] Periodontal diseases are recognized as infectious processes that require bacterial presence and a host response and are further affected and modified by other local, environmental, and genetic factors. The key organisms that cause periodontal disease were anaerobes including *Aggregatibacter actinomycetemcomitans*,*Porphyromonasgingivalis*,*Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, and *Campylobacter rectus*.[3] Periodontitis has been proposed as having an etiological or modulating role in many systemic organ systems like cardiovascular, cerebrovascular disease, diabetes, respiratory disease, and adverse pregnancy outcome; several mechanisms have been proposed to explain or support such theories. One of these is based around the potential for the inflammatory phenomenon of periodontitis to have effects by the systemic dissemination of locally produced mediators, such as C-reactive protein, interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α). Periodontal diseases are recognized as infectious processes that require bacterial presence and a host response which are further affected and modified by other local, environmental, and genetic factors. The oral cavity works as a continuous source of infectious

agents, and its condition often reflects the progression of systemic pathologies.[4] Recent advances in research technology have allowed researchers to study bacteria in their natural environment. Dental biofilm forms via an ordered sequence of events, resulting in structured and functionally organized species-rich microbial community and modern molecular biological techniques have identified about 1,000 different bacterial species in the dental biofilm, twice as many as can be cultured.

Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the bioburden (total microbial load and different pathogenic isolates within that dental plaque biofilm) and maintaining a normal flora with appropriate oral hygiene methods.[5] The ecological plaque hypothesis suggests that selective pressure in environmental conditions can change the balance between oral health and disease. As bacteria can also influence their environment, and both synergistic and antagonistic interactions are suggested for bacteria in dental plaque, the environmental pressure described in the ecological plaque hypothesis could be introduced partly by bacteria.[6] A logical approach to the prevention of periodontal disease is through excellent supragingival plaque control. The toothbrush plays an important role for personal oral hygiene and effective plaque removal.[7] Such control is not generally achieved by mechanical oral hygiene procedures alone. Thus, there is a clear rationale for the use of antiplaque agents to augment mechanical means. Several alcohol-based antiplaque agents are available in the market and the most common is chlorhexidine gluconate, but with scientifically proven side effects associated with chlorhexidine gluconate, i.e., temporary loss of taste; staining of teeth, restorations, and mucosa; dryness and soreness of mucosa; bitter taste; and slight increase in supragingival calculus formation.[8,9] However, changing paradigms in the pathogenesis of periodontal diseases and with the evolution of technology, novel adjunctive antimicrobial approaches, such as probiotics, photodynamic therapy, ozone therapy, antioxidants and local drug delivery, have emerged within the scientific and clinical literature in recent years.[10]

Due to the emergence of antibiotic resistance and frequent recolonization of treated sites with pathogenic bacteria,[11,12] there is need for new treatment paradigms in periodontal disease management. Enormous attention is currently focused for application of probiotics to improve oral health in natural ways. Probiotics being natural and with no side effects have the great potential in suppressing the bacterial proliferation in oral microbiome. According to the generally accepted definition, a probiotic "is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance."[13] World Health Organization (WHO) describes probiotics as "live microorganisms which, when administered in adequate amounts in food or as dietary supplement confer a health benefit on the host."[14] This term has been derived from the Greek language which means "for life." The term probiotic, as an antonym to the term antibiotic, was first used by Lilly and Stillwell in 1965 to describe substances secreted by one microorganism which stimulates the growth of another.[15] The concept of probiotics was brought forward in the first decade of 1900 by a Ukrainian bacteriologist and Nobel Laureate Metchnikoff[16] who observed that bacteria in the fermented milk competed with the microorganisms that are injurious to health.

Recent studies have demonstrated a beneficial health impact of specific probiotic bacteria in humans, leading to several new recommendations for probiotic use to boost entire immune system including oral health. The oral cavity has recently been suggested as a relevant target for probiotic applications. In the oral cavity, probiotics adhere to dental tissues as a part of biofilm, acting as a protective lining for oral tissues against oral diseases. Such a biofilm keeps bacterial pathogens off the oral tissues by filling a space which could have served as niche for pathogens in future and competing with the cariogenic bacteria and periodontal pathogens.[17] Hence, this randomized double-blind placebo controlled clinical study was aimed to evaluate the efficacy of Hyperbiotics PRO-Dental oral lozenges with conventional oral hygiene measure in treatment of mild to moderate chronic periodontitis patients in terms of clinical and microbiological parameter outcomes.

MATERIALS AND METHODS

Patient Selection

The present study was conducted in the Department of Periodontology and Oral Implantology, Pravara Institute of Medical Sciences, Loni, Ahmed Nagar, Maharashtra, India. It was a randomized, double-blinded placebo-controlled clinical study. After an informed consent, a total 40 mild to moderate generalized chronic periodontitis patients between the ages of 18 and 55 years were enrolled in the study and divided under two categories. Each group comprised 20 subjects each, as illustrated in Table 1. Subjects were screened and filtered based on the fixed exclusion criteria as mentioned in Table 2.

Clinical Recording Protocol

Clinical parameters that were evaluated were gingival index (GI), plaque index (PI), probing depth (PD), and

Table 1: Age and sex-wise distribution

	Group I (n = 20)			Group II (n = 20)		
Age in years	*Male*	*Female*	*Total*	*Male*	*Female*	*Total*
<30	0	1	1	0	1	1
30–40	8	4	12	6	5	11
40–50	2	5	7	4	4	8
Total	10	10	20	10	10	20
Mean ± SD	39.70 ± 5.08			40.0 ± 5.00		

Table 2: Study exclusion criteria

- The patient should have a minimum of 20 sound permanent teeth with minimum of 5 teeth to be present in each arch quadrant
- Presence of any systemic neurological disorder (e.g., epilepsy or schizophrenia)
- Presence of a disease with possible effects on the immune system (e.g., chronic infections or cancer)
- Patient who have received antibiotics or nonsteroidal anti-inflammatory drugs (like Ibuprofen) in past 9–11 weeks
- Patients who have received periodontal treatment in past 6 months
- Pregnant and lactating mother
- Patient with artificial prosthesis
- Patients who smokes or consumes tobacco in any form
- Patients suffering from diabetes, arthritis, any type of heart disease like myocardial infarction, coronary artery disease, etc.
- Female patient using intrauterine birth control devices or birth control pills
- Obese individuals (30 and above range as per WHO body mass index cutoff for weight categories for Asians)
- Systemically healthy subjects
- Subjects not willing to participate in the study

Table 3: Study group categorization

Groups	*Scientific protocol*
I	Comprised 20 periodontitis subjects with complete oral prophylaxis followed by conventional oral hygiene measures and supplemented with daily consumption of one Hyperbiotics PRO-Dental probiotic chewable tablet/lozenges at night
II	Comprised 20 periodontitis subjects with complete oral prophylaxis followed by conventional oral hygiene measures and supplemented with daily consumption of one placebo for Hyperbiotics PRO-Dental probiotic chewable tablet/lozenges at night

clinical attachment level (CAL). Patients received a verbal description about the clinical protocol to be followed in this clinical study. In order to have the unbiased and accurate clinical data, we followed a double-blind protocol in the study for enrollment of the patients in terms of treatment plan and further categorization into study group. After enrollment of the subjects in the study, phase 1 therapy (Complete scaling) was done by similar Electro Medical Systems ultrasonic scaler to all the subjects enrolled in the study. All the subjects were advised to brush twice daily 5 minutes with modified bass method technique (technique demonstrated to each subject), and similar medium bristle tooth brushes and tooth paste were provided to each of the subject during the study course to maintain standardization. The subjects (20 each) were then categorized into two treatment regimes in groups I and II as illustrated in Table 3.

Microbiological Recording Protocol

In addition to the clinical study, a microbiological study was also performed from the samples collected from the experimental sites (periodontal pockets). Subgingival plaque samples were from the sample site and immediately transferred into the Robertson's cooked meat transport[18] in a test tube for specific bacterial culturing. The subgingival samples were taken on day 0 (base level), 2 and 4 month recall visit of all the subjects. The key periodontopathic bacteria that were selected are *Aggregatibacteractinomycetemcomitans(Aa),Prevotellaintermedia (Pi), Porphyromonas gingivalis (Pg),* and *Fusobacterium nucleatum (Fn).*

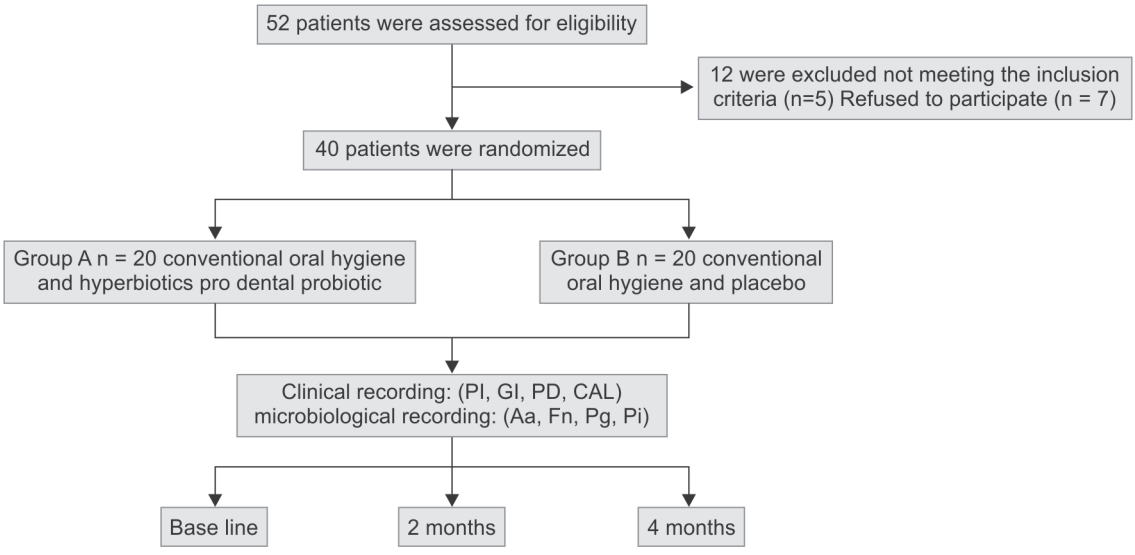
Evaluation Protocol

The design of the study has been illustrated in Flow chart 1. Baseline clinical measurements and microbiological samples were recorded of all the two groups. Phase 1 Therapy (full mouth) was carried out in patients belonging to all the groups, i.e., A and B. Both the probiotic and placebo lozenges could not be discriminated from each other by shape, texture, or taste. The patients were asked to suck one lozenge at night, after tooth brushing and were instructed not to use any probiotic-containing products during the course of the study. Recall visits was scheduled for all the subjects belonging to both the groups (I and II) on 2 and 4 month. (Both clinical and microbiological measurements were recorded.)

Statistical Analysis

For statistical analysis, individual measurements were summarized within each individual and then analyzed. Statistical analysis was performed by applying mean, standard deviation (SD), Student's unpaired t-test, probability (p), analysis of variance, and Tukey–Kramer multiple-comparison tests and Friedman tests.

Flow chart 1: Study design



RESULTS

Clinical Parameters Analysis

Distributions of mean and standard deviation values of all the clinical parameters of both the groups (I and II) were illustrated in Tables 4 and 5. By applying Student's paired t test, there was a highly significant decrease from baseline to 4 months for mean values of clinical parameters (GI, PI, PD, and CAL) in both groups I and II (i.e., $p < 0.01$). By applying Tukey–Kramer multiple comparison test, there was a highly significant difference between mean values of all clinical parameters when compared together at day 0, 2, and 4 months in groups I and II ($p < 0.0001$), where value of $F = 264.63$. By applying Student's unpaired t-test, there was a highly significant difference between mean values of all the clinical parameters in groups I and II at 4 months (i.e., $p < 0.01$). When we compared both these groups, the unpaired t-test value for GI, PI, PD, and CAL was 5.21, 13.74, 3.59, and 2.91 respectively, as illustrated in Table 6. This comparison confirmed that subjects under group I continuously showed more clinical improvements.

Microbiological Parameters Analysis

Distributions of mean and standard deviation values of all the microbiological parameters of both the groups (I and II) were illustrated in Tables 7 and 8 respectively. By applying Student's paired t-test, there was a highly significant decrease from baseline to 4 months for mean values of microbiological parameters *Aa*, *Pi*, *Pg*, and *Fn*) in both groups I and II (i.e., $p < 0.01$). By applying Tukey–Kramer multiple comparison test, there is a highly significant difference between mean values of all microbiological parameters compared together at day 0, 2 months, and 4 months in groups I and II ($p < 0.0001$) where value of $F = 123.05$. By applying Student's unpaired t-test, there was a highly significant difference between mean values of all the microbiological parameters in groups I and II at 4 months (i.e., $p < 0.01$). When we compared both these groups, the unpaired t-test value for *Aa*, *Fn*, *Pg*, and *Pi* was 5.49, 3.23, 4.66, and 3.69 respectively, as illustrated in Table 9. This comparison confirmed that subjects under group I continuously showed more microbiological improvements.

Table 4: Distribution of mean and SD values of clinical parameters in group I

	GI	*PI*	*PD*	*CAL*
Parameters	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Day 0	2.64 ± 0.29	0 ± 0	4.10 ± 0.55	4.15 ± 0.49
2 months	0.81 ± 0.11	0.71 ± 0.11	2.70 ± 0.47	2.80 ± 0.41
4 months	0.68 ± 0.10	0.72 ± 0.11	1.55 ± 0.61	1.85 ± 0.59
Day 0–4 months[ǂ]	45.45	29.26	78.22	78.43
Day 0–2 months[ǂ]	46.11	88.41	189.98	102.81
2–4 months[ǂ]	58.11	28.85	36.72	23.59
p-value	<0.01*	<0.01*	<0.01*	<0.01*

[ǂ]Student's paired t-test value; *Highly significant

Table 5: Distribution of mean and SD values of clinical parameters in group II

	GI	*PI*	*PD*	*CAL*
Parameters	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Day 0	2.57 ± 0.31	0 ± 0	4.10 ± 0.45	4.20 ± 0.52
2 months	1.01 ± 0.11	0.98 ± 0.11	2.95 ± 0.39	2.95 ± 0.39
4 months	1.16 ± 0.04	1.20 ± 0.11	2.10 ± 0.31	2.35 ± 0.49
Day 0–4 months[ǂ]	34.87	48.76	85.68	42.98
Day 0–2 months[ǂ]	23.34	39.82	63.86	275.65
2–4 months[ǂ]	9.58	29.89	47.49	26.82
p-value	<0.01*	<0.01*	<0.01*	<0.01*

[ǂ]Student's paired t-test value; *Highly significant

Table 6: Comparison of mean and SD values of clinical parameters in groups I and II at 4 months

	Group I (n = 20)	*Group II (n = 20)*	*Student's unpaired*	
Clinical parameters	*Mean ± SD*	*Mean ± SD*	*t-test value*	*p-value*
GI	0.68 ± 0.10	1.16 ± 0.04	5.21	<0.01*
PI	0.72 ± 0.11	1.20 ± 0.11	13.74	<0.01*
PD	1.55 ± 0.61	2.10 ± 0.31	3.59	<0.01*
CAL	1.85 ± 0.59	2.35 ± 0.49	2.91	<0.01*

*Highly significant

Table 7: Distribution of mean and SD values of microbiological parameters in group I

	Aa	*Fn*	*Pg*	*Pi*
Parameters	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Day 0	45.30 ± 3.53	46.85 ± 5.33	46.10 ± 4.38	48.45 ± 4.17
2 months	19.75 ± 3.19	21.65 ± 4.77	20.50 ± 3.72	22.60 ± 3.76
4 months	18.25 ± 2.70	20.80 ± 4.87	18.60 ± 3.08	20.45 ± 3.66
Day 0–4 months[ǂ]	335.91	201.15	7.79	13.63
Day 0–2 months[ǂ]	145.68	235.14	6.88	16.21
2–4 months[ǂ]	13.68	37.99	5.94	26.82
p-value	<0.01*	<0.01*	<0.01*	<0.01*

[ǂ]Student's paired *t*-test value; *Highly significant

Table 8: Distribution of mean and SD values of microbiological parameters in group II

	Aa	*Fn*	*Pg*	*Pi*
Parameters	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Day 0	46.45 ± 4.77	45.60 ± 3.76	47.65 ± 3.51	49.30 ± 4.17
2 months	28.85 ± 5.73	28.95 ± 5.85	27.40 ± 5.51	28.75 ± 5.99
4 months	26.15 ± 5.84	26.45 ± 6.11	24.95 ± 5.26	26.15 ± 5.86
Day 0–4 months[ǂ]	81.95	35.61	45.26	50.47
Day 0–2 months[ǂ]	84.80	36.43	57.98	61.23
2–4 months[ǂ]	109.2	42.98	43.81	89.40
p-value	<0.01*	0.01*	<0.01*	<0.01*

[ǂ]Student's paired t-test value; *Highly significant

DISCUSSION

This double-blind placebo-controlled study evaluated the effect of the adjunctive use of Hyperbiotics PRO-Dental probiotics lozenges after scaling and root planing (SRP), one time a day for 4 months, on clinical and microbiological parameters in chronic periodontitis patients. Results of the study clearly marked the advantage of probiotics lozenges on the nonsurgical treatment outcome as compared with placebo group. The results of this study were similar to other studies that incorporated the probiotics to boost oral health in case of periodontitis subjects. Kõll-Klais et al[19] observed that *Lactobacillus gasseri* strains isolated from periodontally healthy subjects were more efficient at inhibiting the growth of *Aggregatibacter actinomycetemcomitans* than strains from periodontally diseased subjects, and also inhibited the growth of *Porphyromonas gingivalis* and *Porphyromonas intermedia*. This correlated with an inverse relationship

Table 9: Comparison of mean and SD values of microbiological parameters in groups I and II at 4 months

Microbiological parameters	*Group I (n = 20)* *Mean ± SD*	*Group II (n = 20)* *Mean ± SD*	*Student's unpaired t- test value*	*p-value*
Aa	18.25 ± 2.70	26.15 ± 5.84	5.49	<0.01*
Fn	20.80 ± 4.87	26.45 ± 6.11	3.23	<0.01*
Pg	18.60 ± 3.08	24.95 ± 5.26	4.66	<0.01*
Pi	20.45 ± 3.66	26.15 ± 5.86	3.69	<0.01*

*Highly significant

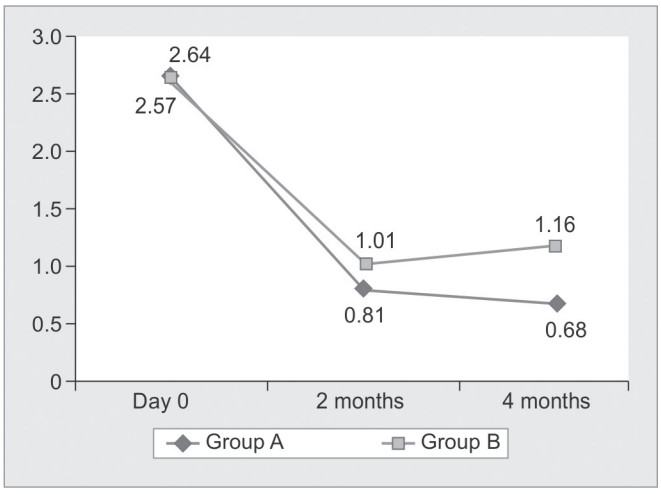
between carriage of homofermentative lactobacilli and subgingival colonization by *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia*. Ishikawa et al[20] observed *in vitro* inhibition of *P. gingivalis*, *P. intermedia*, and *Prevotellanigrescens* by *L. salivarius*. Daily ingestion of *L. salivarius*-containing tablets resulted in reduced salivary counts of these black pigmented anaerobes. Vivekananda et al[21] evaluated the effects of *Lactobacilli reuteri* alone and in combination with SRP in patients with chronic periodontitis for a period of 42 days. Their trial confirmed plaque inhibition, antiinflammatory and antimicrobial effects of *L. reuteri* and they recommended the use of probiotic during nonsurgical and maintenance phase of periodontal treatment. Teughels et al[22] in a randomized placebo-controlled clinical trial that evaluated the effects of *L. reuteri*-containing probiotic lozenges and placebos as an adjunct to SRP in 30 patients with chronic periodontitis, monitored clinically and microbiologically at baseline, 3, 6, 9, and 12 weeks after therapy. Significant improvement in all clinical parameters reduced *P. gingivalis* levels, more pocket depth reduction and attachment gain in moderate and deep pockets were observed in the SRP + probiotic group. Maekawa and Hajishengallis[23] studied whether *Lactobacillus brevis* CD2 or placebo could inhibit periodontal inflammation and bone loss in experimentally induced periodontitis in mice. Mice topically treated with *L. brevis* CD2 displayed significantly decreased bone loss, lower expression of TNF, IL-1β, IL-6, and IL-17A, lower counts of anaerobic bacteria, but higher counts of aerobic bacteria as compared with placebo-treated mice. Hence, *L. brevis* CD2 could inhibit periodontitis through modulatory effects on the host response and the periodontal microbiota.

CONCLUSION

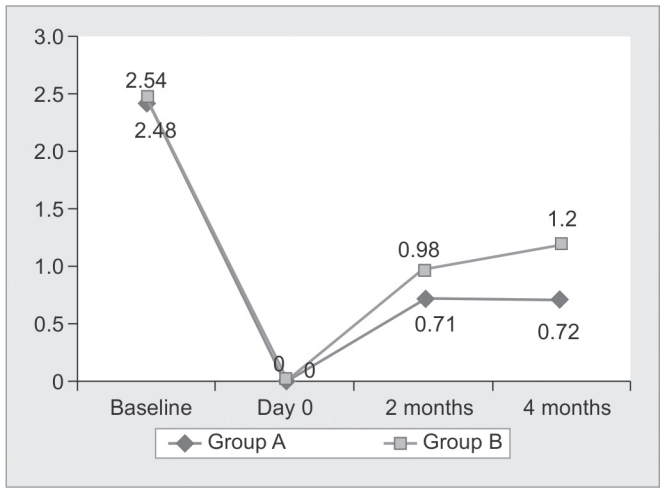
Hyperbiotics PRO-Dental is a patented unique blend of probiotics *S. salivarius (DSM 13084), S. salivarius (DSM 14685), L. reuteri (SD-5865), and L. paracasei (SD-5275)* that can effectively compete with periodontopathic bacteria and lead to repopulate the growth of beneficial bacteria that supports oral health; it not only helps in reducing the pathogenic bacteria count but also suppresses their proliferation as discussed in the study. The fundamental factor that distinguishes it from other oral probiotics is its amalgamation with other key ingredients that synergistically boost oral health as illustrated in Table 10. This study showed that the adjunctive use of hyperbiotics PRO-Dental lozenges resulted in highly significant

Table 10: Key ingredients and relative functions

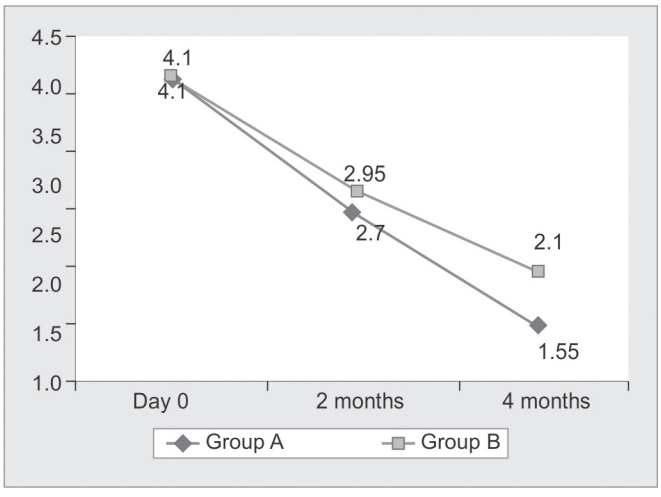
Ingredient	*Property*	*Key advantages*
Zinc	Antioxidant	• Boosts cellular health and connective tissue strength. • Deters the respiration in *F. nucleatum* and other oral microflora to stop the production of reactive oxygen. • Inhibits the production of volatile sulfur compounds, thus assisting in minimizing halitosis. • Inhibits the production of glucosyltransferases in the bacteria and this will affect the colonization of bacterial pathogens and subsequent development and accumulation of dental plaque.
Isomalt	Disaccharide-type polyol	• Dental caries prevention. • Maintains the healthy pH inside oral cavity to prevent demineralization. • Promotes salivary stimulation
Inulin	Ecological balance	• pH balance • Regulates malodor/halitosis • Control of local infection
Dicalcium phosphate	Calculus inhibitor	• Promotes break down of mineralized dental plaque
Stevia	Anti-inflammatory	• Natural sweetener • Antibacterial and antifungal properties • Supports healing



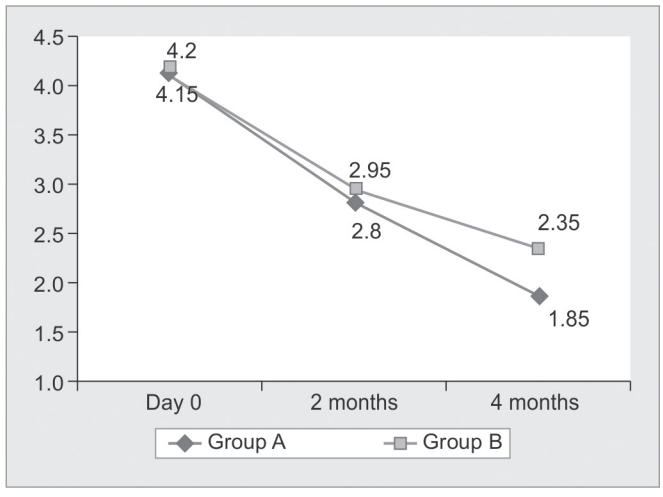
Graph 1: Gingival index – group I *vs* II



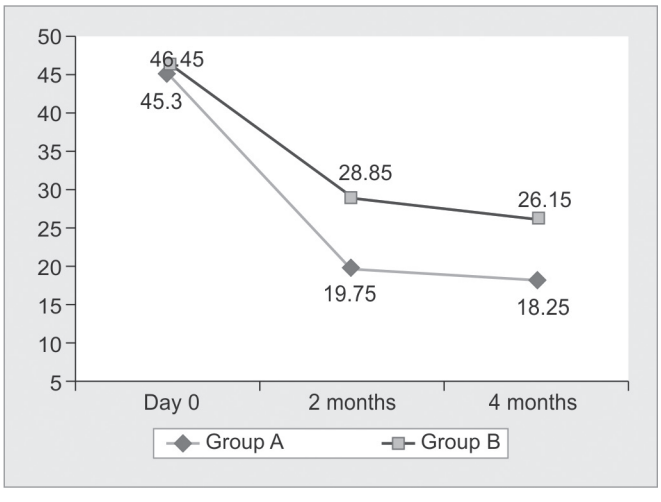
Graph 2: Plaque index – group I *vs* II



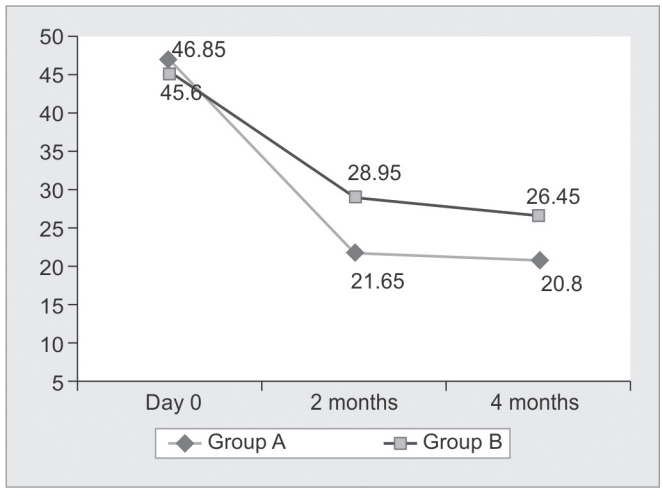
Graph 3: Probing depth – group I *vs* II



Graph 4: Clinical attachment level – group I *vs* II

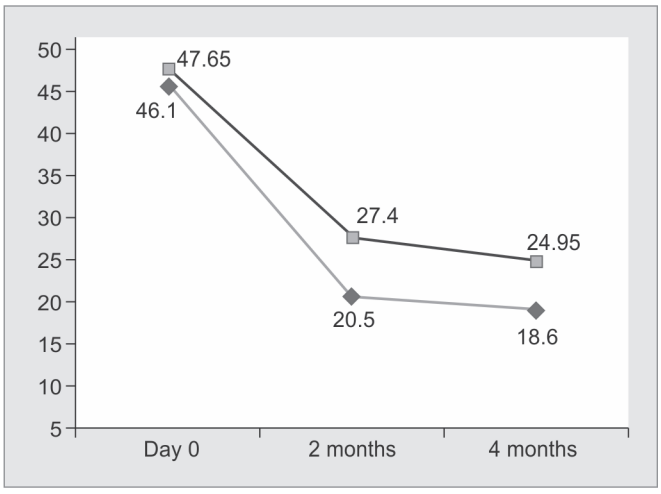


Graph 5: *Aggregatibacter actinomycetemcomitans* – group I *vs* II

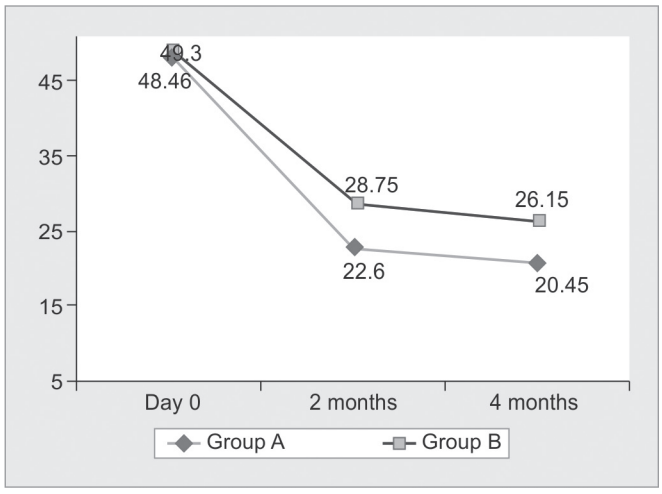


Graph 6: *Fusobacterium nucleatum* – group I *vs* II

additional clinical and microbiological improvements primarily for initially moderate to deep pockets when compared with SRP alone that clearly upheld its potential benefits in long-term maintenance of periodontal health, as illustrated in Graphs 1 to 8. Clinical result of this study showed a clinically essential advantage for the patient as risk for disease advancement, and necessity for additional surgery outcome measures was significantly better when used as an adjunct to SRP. No adverse effect/side effects were reported with subjects under both the



Graph 7: *Porphyromonas gingivalis* – group I *vs* II



Graph 8: *Prevotella intermedia* – group I *vs* II

groups. However, long-term multicentric clinical studies are needed further to establish the complete beneficial effects and truly validate its potential outcomes.

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