LITERATURE REVIEW

Probiotic therapy as a novel approach in the prevention and treatment of gingivitis. A review

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ABSTRACT

Objective: Gingivitis is caused by a mixture of Gram-positive and Gram-negative species and is characterized by inflammatory exudate in the marginal region. Probiotics have been introduced in the prevention and treatment of periodontal diseases and represent an innovative approach to maintaining oral health using beneficial bacteria when administered in adequate amounts. The mechanism of action of probiotics in the oral cavity is not fully understood but is commonly explained by a combination of local and systemic immunomodulation. The results of the studies suggest that probiotics may be useful in the prevention and treatment of gingivitis, but their effectiveness is still very questionable. Thus, more in vivo research is needed through randomized controlled studies evaluating the main periodontal clinical parameters, levels of inflammatory mediators and microbiological analyzes, especially to elucidate some still unknown mechanisms of action and to ensure the use of probiotics as an effective therapy in the prevention and treatment of gingivitis.

KEYWORDS

Gingivitis; Probiotics; Periodontal treatment.

RESUMO

Objetivo: A gengivite é causada por uma mistura de espécies Gram-positivas e Gram-negativas e é caracterizada pelo exsudado inflamatório na região marginal. Os probióticos tem sido introduzido na prevenção e tratamento das doenças periodontais e representam uma abordagem inovadora para manter a saúde bucal através da utilização de bactérias benéficas quando administradas em quantidades adequadas. O mecanismo de ação dos probióticos na cavidade bucal não é totalmente compreendido, mas é comumente explicado por uma combinação de imunomodulação local e sistêmica. Os resultados dos estudos sugerem que os probióticos podem ser úteis na prevenção e tratamento da gengivite, porém sua eficácia é muito questionável ainda. Desta forma, são necessárias mais pesquisas in vivo através de estudos randomizados controlados avaliando os principais parâmetros clínicos periodontais, níveis de mediadores inflamatórios e análises microbiológicas, especialmente para elucidar alguns mecanismos de ação ainda desconhecido e para assegurar a utilização dos probióticos como terapia eficaz na prevenção e tratamento da gengivite.

PALAVRAS-CHAVE

Gengivite; Probióticos; Tratamento periodontal.
INTRODUCTION

Probiotic is defined as “live microorganisms which, when administered in adequate amounts, confer beneficial effects to the hosts health” [1]. Probiotics are available in different foods and dietary supplements [2] and traditionally the main research field is associated with the gastrointestinal tract [3]. However, during the last decade, several studies suggest that probiotics may also be beneficial for oral health [3,4], however the mechanisms of action are not fully known.

The therapeutic effects for prevention and treatment of periodontal disease (PD) can be proposed through direct interactions within the dental plaque, such as interruption of dental biofilm formation by competition for binding sites in tissues of the host, other bacteria and competition for nutrients, production of antimicrobial compounds that inhibit oral bacteria, as well as lactic acid bacteria produce antimicrobials as organic acids and hydrogen peroxide, and actions indirect within the oral cavity, including the modulation of innate and specific immune response [5].

The goal of periodontal therapy is prevention and treatment of periodontal inflammation and establishment of a beneficial or biofilm associated to health [6]. Evidence shows that the mechanical removal of the supragingival plaque is the most effective tool to control the plaque and prevent gingivitis [7]. However, most individuals fail to adequately control the accumulation of plaque and gingivitis is still highly prevalent [8,9]. In this way, antimicrobial products have been tested to assess your effectiveness in reducing plaque and adjuvant gingivitis [10], and the increase of antibiotic resistance is a reason to seek other treatment also infections in the oral cavity. In this way, are proposed as adjuvants probiotic therapy methods.

A positive effect of the use of probiotics in gingival indices [11-14] and the accumulation of plaque have been demonstrated repeatedly [11,12,15] and your use to combat gingival inflammation was described by several authors [2,11,13,15-19].

Thus, the objective of the present study was to conduct a review of the studies that used the therapy with probiotics for the prevention and treatment of gum disease in humans, to assess the effectiveness of this therapy.

REVIEW OF THE LITERATURE

Probiotics in the prevention and treatment of gingivitis

Studies on the use of probiotics in the prevention and treatment of gingivitis are still very scarce and incipient. One of the clinical studies, we can highlight of KRASSE et al. (2006) [11] that evaluated whether the Lactobacillus reuteri was effective in the treatment of gingivitis. In this randomized, double blind, placebo controlled 59 individuals with moderate to severe gingivitis received two different formulas containing Lactobacillus reuteri or placebo. At the beginning of the study, the gingival and plaque indices were measured in two areas. Patients received instructions on brushing and flossing use before the beginning of the use of probiotic. After 14 days, the gingival index (GI) has decreased in the three groups. The plaque index (PI) decreased significantly in the groups that used the probiotic, but there was no significant difference in the placebo group. This study demonstrated that the probiotic L. reuteri was effective in reducing plaque and gingivitis both in individuals with moderate to severe gingivitis.

Staab et al. (2009) [2] have assessed if a probiotic milk drink containing Lactobacillus casei strain Shirota, was effective on gingival
health and the development of experimental gingivitis. In this parallel study, not blind 50 student volunteers received daily 65 ml of probiotic milk drink (Yakult, Honsha Co., Tokyo, Japan) or not, for 8 weeks and after, individual control of mechanical plate was interrupted during 96 h. At the beginning of the study, the papilla bleeding index, index of interproximal plaque and plaque index (PI) were measured and gingival fluid collected for analysis of polymorphonuclear elastase, myeloperoxidase (MPO) and metalloproteinase-3 (MMP-3) after 8 weeks and again after 96 h. The interproximal plaque index and papillary bleeding index were not different between the groups. In the test group, the activity of elastase and the amount of MMP-3 were significantly lower after the ingestion of probiotic milk drink. This pilot study has shown that the probiotic Lactobacillus casei had a beneficial effect on periodontal health.

Twetman et al. (2009) [13] evaluated the effect of chewing gum containing the probiotic Lactobacillus reuteri (ATCC 55730 e ATCCCPTA 5289) atadoseof1x10^8 UFC/gum (Biogaia) in gingival fluid inflammatory markers in individuals who had chronic gingivitis. The levels of IL-1 β, TNF-α, IL-6, IL-8 and IL-10 were rated 1, 2 and 4 weeks after the start of the study. Patients received instructions on brushing and flossing use before the beginning of the use of probiotic. The participants were instructed to chew actively one lozenge of chewing gum for 10 minutes twice a day. The levels of TNF-α and IL-8 were significantly reduced in the group that used the probiotic twice a day after the first and second weeks. In the fourth week, there was no difference in the levels of inflammatory markers. In the group which used chewing gum containing probiotic once a day, there was no difference in inflammatory markers. This study demonstrated that the probiotic Lactobacillus reuteri promotes a reduction of pro-inflammatory cytokines in the gingival crevicular fluid and may combat inflammation in the oral cavity.

Harini et al. (2010) [20] evaluated clinically if a mouthwash containing probiotic was effective on the gingival plaque accumulation in children. In this experimental study double-blind, comparative of 14 days with 45 healthy children between the ages of 6-8 years, comparing the mouthwash containing chlorhexidine and probiotic, were instructed to rinse 1 time a day about to 30 min after brushing with 15 mL the solution for 60 seconds. The probiotic group and chlorhexidine showed reduced accumulation of plaque compared to the control group at the end of 14 days. However, unlike the plaque index (PI), there was significant difference in gingival index (GI) between the probiotic group and chlorhexidine. The probiotic group was better than the Chlorhexidine group. The data suggest that the mouthwash probiotic has been effective in reducing plaque accumulation and gingivitis, having a potential therapeutic effect.

Slawik et al. (2011) [17] evaluated if a probiotic milk drink containing Lactobacillus casei strain Shirota, was effective during the various stages of gingivitis induced by plaque. In this prospective clinical controlled study, 28 adults with healthy gums, received daily 65 ml of probiotic milk drink (Yakult, Honsha Co., Tokyo, Japan) containing 6.5 billion living strains of Lactobacillus casei Shirota for 4 weeks; the control group did not receive any probiotic food or drink. After 2 weeks of probiotic drink consumption, participants were advised not to brush your teeth for 14 days. Later, both at the beginning as in days 1, 3, 5, 7 and 14, were evaluated the clinical parameters of plaque index (PI), gingival index (GI), gingival crevicular fluid volume (GCF) and bleeding on probing (BOP).
interruption of oral hygiene increased clinical inflammatory parameters in both groups. On day 14, the IP, IG, volume of GCF and BOP parameters were significantly higher compared with the baseline values. On day 14, the BOP levels and the volume of GCF were significantly lower in the test group compared with the control group. The data suggest that a daily consumption of a probiotic milk drink reduces the effects of gingival inflammation induced by plaque.

Iniesta et al. (2012) [18] have evaluated if chewable tablet containing the probiotic Lactobacillus reuteri given orally was effective in oral microbiota. In this parallel, placebo-controlled study, 40 patients with gingivitis, received 1 chewable tablet daily for 28 days, containing Lactobacillus reuteri DSM-17938 and ATCC PTA 5289) a dose of 2 x 10^8 CFU/pill or placebo. Clinical and microbiological analyses were carried out at baseline, 4 and 8 weeks. In the saliva, total anaerobic counts after 4 weeks and Prevotella intermedia counts after 8 weeks, showed reductions in the test group. Subgingival samples, significant reductions were observed in the change in baseline to 4 weeks for Porphyromonas gingivalis counts. This study demonstrated that pills of Lactobacillus reuteri administered resulted in a reduction in the number of selected in subgingival microbiota periodontal pathogens.

Karuppaiah et al. (2013) [15] evaluated if the probiotic was effective in reducing plaque. In this randomized, double-blind, placebo-controlled study, 216 students (14-17 years) were divided into testing groups including curd in your daily diet for 30 days, while the controls ruled out the curds in your diet for 30 days. The plaque index (PI) and gingival index (GI) post-intervention were registered and statistically compared with the baseline data. It was found that the intervention group had a statistically significant reduction in the plaque when compared with the control group and there was no significant improvement in gingival health. This study showed that the short-term daily intake of probiotics via dietary curd reduced the levels of plaque, the data suggest that could improve oral health.

Hallström et al. (2013) [19] evaluated if daily oral administration of lozenges containing probiotic Lactobacillus reuteri could influence the inflammatory response and the composition of the supragingival plaque. In this crossover study double-blind, placebo-controlled in experimental model of gingivitis in 18 healthy women, the vestibular surface of the first molars was used as experimental locations. A mouth guard that covered the first premolar to second molar was used when brushing, avoiding an accidental cleaning for 3 weeks of plaque build- up. Lozenges containing Lactobacillus reuteri (ATCC 55730 e ATCC PTA 5289) or placebo were administered twice daily for 21 days. At baseline and after 21 days, plaque index (PI), gingival index (GI) and bleeding on probing (BOP) were recorded. Gingival crevicular fluid (GCF) samples were analyzed for the concentration of 7 inflammatory mediators. No differences were observed between the test and placebo. Likewise, the microbial composition did not differ between groups. This study showed that the daily intake of probiotic lozenges did not appear to significantly affect plaque buildup, inflammatory reaction or biofilm composition during experimental gingivitis.

Lee et al. (2015) [21] evaluated if the probiotic Lactobacillus brevis CD2 was effective in anti-inflammatory properties, preventing the synthesis of nitric oxide. In this randomized study, 34 healthy adults received Lactobacillus brevis CD2 lozenges or placebo 3 times daily for 14 days. In both groups bleeding on probing (BOP) increased continuously over the course of the study, except on day
3. In the placebo group, scores increased significantly from 9.50 at baseline to 14.75 and 14.81 on days 10 and 14, respectively. However, scores were consistently higher with placebo, and significant intergroup differences were observed on day 10. Plaque index (PI) and gingival index (GI) increased at baseline in both treatment groups. Measurements of immunological markers in gingival crevicular fluid (GCF) revealed increased nitric oxide production in the placebo group. This study demonstrates that *Lactobacillus brevis* CD2 may delay the development of gingivitis in this model by regulating the inflammatory cascade.

Toiviainen et al. (2015) [22] evaluated whether probiotics *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium animalis* subsp. Lactis BB – 12(BB-12) were effective on the number of mutans salivary streptococci, plaque amount, gingival inflammation and oral microbiota. In this randomized controlled, double-blind study, 77 healthy young adults received lozenges containing a combination of LGG and BB-12 or lozenges without addiction of probiotics for 4 weeks. The probiotic *Lactobacillus rhamnosus* GG (ATCC 53103) (Probiotical S.p.A., Novara, Italy), and *Bifidobacterium lactis* BB-12 (DSM 15954) (Chr, Hansen A/S, Hoersholm, Denmark) on daily dose of 2x10⁹ cells for LGG and BB-12 each. At the beginning and at the end of de period plaque index (PI) and gingival index (GI) were determined, and the stimulated saliva was collected. The probiotic lozenge decreased PI and GI while no changes were observed in the control group. However, no probiotic-induced changes were found in salivary microbial compositions in any of the groups. This study demonstrated that the probiotic lozenges improved the probiotic status without affecting the oral microbiota.

Kolip et al. (2016) [23] evaluated the effects of probiotics on plaque accumulation and gingival inflammation in subjects with fixed orthodontics. In this pilot study, 15 healthy patients aged 11-18 years undergoing fixed orthodontic treatment used a fully dissolved and natural lozenges containing six probiotic strains (Dentaq® Oral and ENT Health ProbioticComplex) for 28 days. Clinical measurements were performed at baseline (day 0) and at the end of probiotic use (day 28), evaluating gingival index and plaque index. They observed that in the baseline, plaque index and gingival index decreased 28.4% and 35.8% in each patient, respectively, on day 28, thus reporting a decrease in dental and gingival pain, decreased bleeding and increased gingival index. motivation to maintain adequate oral hygiene throughout the study. This study provided preliminary support for the use of the Dentaq® Oral Probiotic Complex and ENT Health Probiotic as a safe and natural product for the reduction of plaque accumulation and gingival inflammation.

Alkaya et al. (2017) [24] evaluated whether 3 different modes of probiotic applications were effective in the oral cavity. In this double-blind, placebo-controlled, randomized study, 40 patients with generalized gingivitis aged 18-31 years used a probiotic toothpaste, probiotic mouthwash, and a box to clean the toothbrush containing *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus pumulus* in concentration of 5x10⁷ UFC / spores of *B. subtilis*, *B. megaterium* and *B. pumulus* versus placebo. Clinical measurements were performed at the baseline and after 8 weeks of probiotic or placebo use, attachment loss and gain, probing depth(PD) and bleeding on probing (BOP). The patients performed brushing twice a day using the toothpaste, and each night, before bedtime, they used the mouthwash for 1 minute. This study did not show statistically significant differences between a group of patients who used a probiotic toothpaste, a mouthwash and a toothbrush cleaner.
Sabatini et al. (2017)[25], evaluated whether a combination of probiotics (*Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289) was effective in the treatment of gingivitis in diabetic patients. In this double-blind randomized controlled study, 80 adult patients with a diagnosis of controlled type 2 diabetes mellitus and gingivitis were divided into 2 groups. Only oral hygiene instructions were given. Patients in the probiotic group were instructed to consume probiotic lozenges (Reuterinos®) 2 times a day after brushing. Clinical measures of plaque index and bleeding on probing were performed at the beginning and after 30 days. The mean differences between the PI and BOP during the baseline did not show a statistically significant difference between the groups, while the difference between the groups was statistically significant after the 30-day period (*p* <0.005). The study demonstrated that the consumption of probiotics with *Lactobacillus reuteri* produces a significant reduction of PI values and gingival index in type II diabetic patients with gingivitis.

Montero et al. (2017) [26] evaluated the efficacy of a probiotic combination (lozenges) in the treatment of gingivitis and its impact on the subgingival microbiota. In this placebo-controlled clinical study, 59 patients with gingivitis aged 18-55 years were divided into 2 groups. The placebo group and the test group receiving lozenges with *L. plantarum* CECT 7481 (AB15), *L. brevis* CECT 7480 (AB38), and *P. acidilactici* CECT 8633 (AB30) at the dosage of 1x10³ colony forming units (CFUs) for each probiotic strain that should be chewed 2 times a day (morning and evening) after oral hygiene procedures for 6 weeks. Both groups at day 0 received mechanical removal of professional plaque (PMRP: “professional mechanical removal of supragingival plaque” and calculus with subgingival debridement to the depth of the sulcus / pocket). Microbiological samples and clinical parameters [gingival index (GI), plaque index (PI), angles bleed score (AngBS)] were evaluated in 4 sites (distobuccal, mesiobuccal, buccal and lingual) in all teeth after 6 weeks. The results of this study showed that the use of lozenges containing probiotic strains of *L. plantarum*, *L brevis* and *P. acidilactici* were able to reduce gingivitis when used in conjunction with PMPR.

Kuru et al. (2017) [27], evaluated the effect of using 4 weeks of yogurt supplemented with *Bifidobacterium animalis subsp. lactis* DN-173010 versus placebo yogurt, followed by a 5-day non-brushing period. In this randomized controlled clinical study, 51 periodontally healthy patients (19 male and 32 female) aged 16 to 26 years were divided into two groups: placebo and probiotic. During a 28-day period (4 weeks) patients consumed 110g of placebo (no probiotic) or yogurt containing probiotic (containing ≥ 10⁸ CFU / g *Bifidobacterium lactis* DN-173010) daily. It was recommended that the product be used in the morning between breakfast and lunch and that they should not feed or brush their teeth for at least 1 hour after consumption of the yogurt. The period of consumption of yogurt was followed by a period of 5 days of plaque accumulation in which patients should abstain from any oral hygiene measures. Seven days before the start of the study participants received verbal reinforcements on oral hygiene and professional tooth cleaning was performed with abrasives and brushes. The clinical measurements (PI, GI, PD and BOP) and collected gingival crevicular fluid (GCF) samples for immunological analysis were performed on day 0, day 28 (end of the yogurt consumption period) and day 33 (end of period of non-brushing). The study demonstrated a reduction in the clinical and immunological signs of inflammation in a
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non-brushing model after a 28-day period of consumption of yogurt containing \(10^8\) CFU / g of *B. animalis subsp. lactis*. These effects could be seen at the clinical level (PI, GI, BOP and PD) and for GCF markers (GCF volume, IL-1β concentration and total amount of IL-1β).

Arat Maden et al. (2017) [28] evaluated whether a commercially available fluoride, xylitol or xylitol-probiotic dentifrice were effective in reducing plaque and gingival inflammation in children between 13 and 15 years of age. In this prospective, randomized, placebo-controlled clinical study, 48 adolescents were grouped into three groups of n = 16 each. Group A received xylitol (Xyliwhite) toothpaste; group B received xylitol-probiotic dentifrice (PerioBiotic); and control group C received fluoride toothpaste (Colgate Max Fresh). Patients were instructed to use the determined toothpaste and a modified Bass brushing technique twice a day for two minutes over a period of 6 weeks. Clinical measurements were performed at baseline and after 6 weeks evaluating gingival index and plaque index. They observed that after 6 weeks there was a statistically significant reduction with respect to plaque index in all groups respectively. Regarding the gingival index there was a statistically significant reduction in the Colgate Max Fresh and PerioBiotic groups. However, statistically significant differences with PerioBiotic and Colgate Max Fresh were not observed. This study demonstrated that PerioBiotic was a dentifrice that achieved a significant reduction in both gingival index and plaque index.

Keller et al. 2018 [29], evaluated the clinical and microbiological effects of probiotic strains in patients with moderate gingivitis. In this randomized placebo-controlled study, 47 adult patients received tablets containing a blend of *Lactobacillus rhamnosus* PB01, DSM 14869 and *Lactobacillus curvatus* EB10, DSM 32307 or placebo over the 4-week period. Clinical measurements were performed at baseline and after 2, 4 and 6 weeks evaluating, bleeding on probing, plaque index and gingival crevicular fluid flow in which the concentration of cytokines [interleukin (IL) -1β, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α)] through multiplex. In the probiotic group there was a significant reduction in bleeding on probing and in the amount of gingival crevicular fluid after 4 weeks when compared to the baseline. There was no difference between the concentration of the cytokines. The occurrence of both probiotic strains increased in the saliva of test subjects during the intervention but returned to baseline levels within 2 weeks.

This study demonstrated that there was a marked improvement in gingival health in the probiotic group.

**DISCUSSION**

The aim of this review was to determine if the use of probiotics has benefits in the prevention and treatment of gingivitis. The available evidence does not allow a clear statement on the efficacy of probiotics in the prevention and treatment of gingivitis because different trials show still controversial results.

It can be verified that no study evaluates all clinical parameters, levels of inflammatory mediators and microbiological analyzies, which makes it difficult to compare the results of the studies. However, the clinical parameters of PI, GI, PD and BOP were the most evaluated in the studies, although some studies have evaluated inflammatory mediators in the GCF [2,13,17,19,21,27,29] and microbiological parameters [18,22,26] showing beneficial results, but it does not allow to make a clear statement about the efficacy of this intervention.

The studies evaluated in this review
also showed a great variability in the type of probiotics used, doses of probiotic used, time of administration, and different forms of administration of probiotics. However, it seems that in the studies evaluated, the main probiotic strains that were used are the lactic acid bacteria belonging to the genus Lactobacillus. Whereas they have an antagonistic growth property, they are beneficial as bioprotective agents for the control of infection and offer protection against invasive pathogens [30].

Some studies have evaluated the effects of probiotics in the form of a milky drink, demonstrating that it is effective on gingival health [2,17] for 8 or 4 weeks, using curds in their diet [15] or consuming yogurt [27] since they are the most natural way of oral administration. Several authors [13,18,19,21,22,23,25,26, 29] have used lozenges or chewing gum, observing results that can combat inflammation in the oral cavity, such as reduction of periodontal pathogens, reduction of PI, reduction of BOP and reduction of proinflammatory cytokines. Thus, probiotics can influence oral health, modulating the immune response, influencing the oral microbiota, the production of antimicrobial substances, modifying the oral microbiota, competitive exclusion and preventing the adhesion of oral pathogens [31].

Sabatini et al. (2017) [25] demonstrated that the use of probiotic tablets has a positive effect also in patients with systemic diseases, such as Type II Diabetes Mellitus with gingivitis with reduction in plaque index and gingival index. Thus, these results are important because these patients are more likely to develop infections such as gingivitis and the use of probiotics may influence the control of oral biofilm alteration, inhibiting the growth of pathogenic bacteria and Spinler et al. (2014) [32] reported an anti-inflammatory effect by inhibiting the production of TNF-α.

Harini et al. (2010) [20] used a probiotic mouthwash and demonstrated a reduction of plaque accumulation and gingival inflammation, presenting a potential therapeutic effect. Whereas mouthwashes act by reducing non-specifically the levels of beneficial and harmful oral bacteria, in this way they promote a healthy balance of microorganisms in the oral cavity.

Regarding the dose and frequency of the use of probiotics is another factor that we should consider. According to Twetman et al. (2009) [13], it can be observed that the probiotic used twice a day reduced levels of TNF-α and IL-8 significantly compared when administered once a day. Lee et al. (2015) [21] found that administration of probiotic three times daily delayed the development of gingivitis in this model by regulating the inflammatory cascade.

Regarding the time of use, it can be verified that when the probiotic was used for 28 days there was a reduction in the number of periodontal pathogens [19]. This demonstrates a positive effect on the control of gingivitis in relation to the frequency of probiotic use, since probiotic species do not reside permanently in the oral cavity [3,31]. In this way Keller et al., 2018 [29] observed that the occurrence of probiotic strains (Lactobacillus rhamnosus PB01, DSM 14869 and Lactobacillus curvatus EB10, DSM 32307) increased in the saliva of subjects who used the probiotic-containing tablet throughout the intervention but returned to baseline levels within 2 weeks. Therefore, the authors do not know for sure the ideal dose required to obtain satisfactory results in the oral cavity [3,31].

In this way, probiotic bacteria accumulate in microbial biofilms, replacing or reducing pathogenic bacteria [33,34], which may have immunomodulatory effects on the gingival region [2].
CONCLUSION

It can be concluded that the use of probiotics in periodontal treatment is a very current issue and is emerging as a potential therapy. This review has demonstrated that numerous studies have shown satisfactory effects in the prevention and treatment of gingivitis in the supragingival plaque control, but with results still controversial. Therefore, more in vivo studies are needed through randomized controlled trials evaluating the main periodontal clinical parameters, levels of inflammatory mediators and microbiological analyses specially to elucidate some mechanisms of action still unknown and to ensure the use of probiotics as an effective therapy in the prevention and treatment of gingivitis.

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