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Comparative Evaluation of 3 Commercial Mouthwash Formulations on Clinical Parameters of Chronic Gingivitis

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Background: Chlorhexidine (CHX) is not prescribed as a mouthwash for long-term use; therefore, probiotic/herbal mouthwashes are being investigated. This study compared the effect of 3 commercial mouthwashes on plaque index (PI), gingival index (GI), and bleeding index (BI) in patients with chronic gingivitis.

Material/Methods: Forty-five patients (all with moderate plaque) were randomly allocated into 3 groups (Gp): Gp 1 (CHX), Gp 2 (Manuka), and Gp 3 (Pro-Dental). Three periodontal clinical parameters – PI, GI, and BI – were recorded at baseline and on days 7, 14, and 28. An oral hygiene maintenance program was followed by a double-blinded intervention (coded bottle containing mouthwash). Both inter-group and intra-group comparisons were made using analysis of variance (ANOVA) with multiple *t* tests. All probable values were considered to have various levels of significance at $P<0.05$ or below.

Results: All indices for all groups showed higher values (mean) at baseline, which were lower on days 7, 14, and 28. No differences in any clinical parameter at any point of time existed between Gp 1 and Gp 2. There were, however, significant differences ($P<0.05$) between Gp 1/Gp 3 and Gp 2/Gp 3 for all clinical parameters at all observed time periods (days 7, 14, 28). Intra-group comparison for all groups demonstrated highly significant differences between baseline values and other time points.

Conclusions: For managing chronic gingivitis, Manuka mouthwash is as effective as a CHX mouthwash, as there were no differences observed in any clinical parameters at any point points.

Keywords: Biofilms • Dental Plaque • Dental Scaling • Gingivitis • Mouthwashes

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Background

The periodontium plays an essential part in defense of teeth against occlusal forces, bacterial invasion, and stress concentration. Common diseases like gingivitis and periodontitis associated with the periodontium of natural teeth are predominantly inflammatory in nature and originate mainly from microbes [1]. Besides multiple risk factors, accretion of plaque biofilm in and around the junction between tooth and gingiva regularly initiates a host inflammatory immune response that in some cases may even be maladaptive and damaging [2]. Irrespective of the severity of the host response, the control of plaque (mechanical or chemical) around the tooth gingiva junction is necessary in preventing and managing development of periodontal disease [3]. Use of mouthwash is considered as an adjunct to primary therapy, which is the mechanical or manual removal of dental plaque using a toothbrush or other aids. Controlling gingivitis is essential to prevent the development of periodontal disease. Evidence suggests that mouthwash used alone reduces mild gingivitis within 4-6 weeks, but is ineffective if gingivitis is moderate or severe, even after 6 months of use. Mechanical removal of plaque in such cases is necessary before prescribing mouthwash [4]. In patients with specific physical disability/psychological conditions or in other medically compromised patients (eg, hemiplegia or frail patients) who have limited dexterity, adjunctive use of chemical agents in addition to primary mechanical removal is considered mandatory [4]. These patients' ability to follow medical instructions under normal conditions has been established to be meager, thus affecting treatment compliance and treatment satisfaction [5]. Within dentistry, a patient undergoing orthodontic fixed appliance treatment or having a fixed implant prosthesis is more dependent on chemical cleaning rather than physical self-removal of plaque [6]. Thus, mouthwashes play a pivotal role in supragingival plaque control.

Chlorhexidine (CHX) is a salt form of chlorhexidine gluconate/acetate, which is basically a disinfectant and antiseptic. It has long been considered as a scientifically reliable antimicrobial oral mouthwash that has proven long-term efficacy [7]. CHX, being a biocide (broad-spectrum), inactivates by disruption of cell membranes within a short period of time. The cell wall sites (negatively charged) easily bind with its molecule (positively charged), which leads to interference with cell osmosis [8]. Besides its beneficial effects, dysgeusia, tooth stains, dehydration, and painful mucosa limit its long-term use [9]. This has prompted recent research to focus on biological and herbal mouthwashes, for better oral health maintenance, owing to their wide range of biological and medicinal activities, ease of availability, higher safety margins, and lower cost [10]. Biological agents such as probiotic mouthwash increases the commensal flora, thereby preventing the microbiological shift and colonization of pathogens associated with gingival inflammation [11]. Increasing the normal oral microbiota ("good" bacteria) shifts the balance from disease to health and overpowers the ill effects of other bacteria ("bad" bacteria), a mechanism seen in the gut. The probiotic mouthwash formula may either contain strains of Streptococcus or Lactobacillus. These microorganisms are part of the average oral microbiota that occur naturally in the mouth and, when introduced by supplements, can adhere to mucosa easier than other microorganisms and hence colonize the mouth. They can reduce plaque formation, thereby improving overall gingival health [12]. Zinc in addition to bacteria is a powerful antioxidant that plays a role in scavenging free radicals and thereby maintaining periodontal health [13]. These probiotic-based formulations encapsulate bacteria to ensure viable bacteria, which is responsible for its longer shelf life.

Herbal agents that are obtained from plants (chemotherapeutic and medicinal) have also been introduced, which behave as drugs due to the presence of bioactive compounds. Various bioactive compounds (phenolic compounds - flavonoids and phenolic acids) introduce antibacterial properties and therefore have become a substitute for conventional biochemical mouthwashes [14]. Manuka mouthwash contains ingredients that are high nutritive sources of ascorbic acid (vitamin C) and other antioxidants (eg, lutein, alpha linolenic acid, omega-3 fatty acid) [15]. The antioxidants are free scavengers that help in maintaining the integrity of periodontal tissues. Vitamin C is a potent antioxidant that is considered essential for maintaining the integrity of connective tissue, osteoid tissues, and dentine [15]. The antibacterial property of Manuka honey is associated with its sugar content (high). It also inactivates the bacteria through an osmotic effect (phenolic and antioxidant action). Its action is dependent on one of its enzymes (inhibin), which produces hydrogen peroxide on dilution [16]. Generally, studies have shown that mouthwashes either reduce or inhibit bacterial growth (mean bacterial count) with various mean reductions after 2 weeks of use [17].

With the above background, this randomized control trial (RCT) was aimed to evaluate the clinical effectiveness of herbal, probiotic and chlorhexidine mouthwash and their effect of various clinical parameters in healthy patients with dental biofilm-induced gingivitis. The aims of the study were to find a long-term alternative to the commonly used chlorhexidine mouthwash. The objective of the study was to substitute the commonly prescribed CHX mouthwash with a natural alternative that is more biocompatible with oral tissues and does not cause burning, dysgeusia, or tissue dehydration as short-term outcomes and tooth staining as the long-term outcome.

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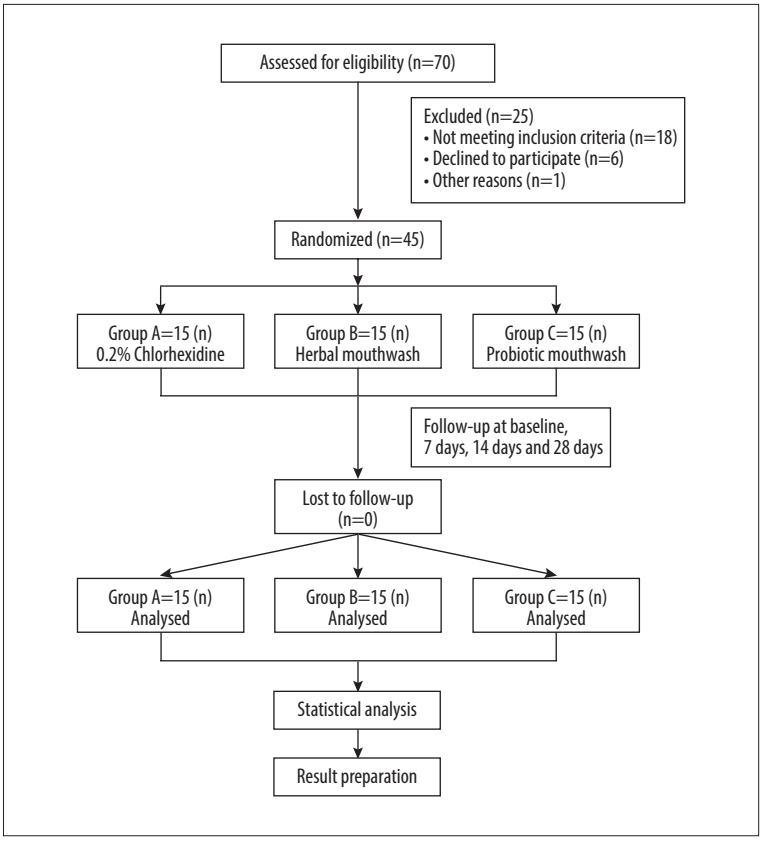

Figure 1. Flow chart (graphical presentation) of the selection of study participants. *Figure created using MS word, 20H2 (OS build 19042,1466), Windows 10 Pro, Microsoft corporation).*

Material and Methods

Ethics

This clinical study was conducted between the second and fourth quarter of 2018 in a dental institute situated in the southwestern region of Saudi Arabia. The study plan was permitted by the college and the university ethics committee under ethical clearance number SRC/ETH/2017-18/081, which conducts all clinical studies involving humans and animals according to the standards and regulations of the Helsinki Declaration.

Patients who visited the outpatient department (OPD) of the college are required to provide a written consent for any diagnostic, preventive, or treatment procedure as a protocol set by the college and the university.

Study Design

This study was a double-blind randomized controlled trial (RCT) in which the participants (patients) and the investigators who were assessing the outcome were not aware of the intervention. The study utilized a prospective, exploratory, comparative approach on a cross-sectional population sample of people who sought periodontal treatment in the OPD.

Sample Size Selection, Treatment, and Grouping

The graphical depiction of the study and the sequence of events are presented in **Figure 1**. The total sample size that would satisfy the research requirements (flexibility, efficiency, and reliability) was calculated to be 45 patients [±5% accuracy, an alpha of 0.05 (95% confidence interval)]. A total of 70 patients were evaluated for preparing the study sample, obtained from the OPD of the Department of Periodontology.

Local and systemic factors that influence plaque deposition formed the basis of inclusion and exclusion criteria. We included patients aged 20-40 years, with a complete set of permanent dentition, with no history of tooth loss, orthodontic, orthognathic or restorative treatment, presence of class 1 molar and canine relation with no evidence of spacing or crowding, ideal proximal contacts, cooperative, with chronic moderate plaque (biofilm) induced gingivitis (mild and/or moderate gingivitis), and not taking any current medication. We excluded patients with a history of systemic disease that would influence periodontal status, smokers, khat chewers, pregnant and lactating women, recent antibiotic use (≤1 month), and having undergone any periodontal treatment in the past 6 months. All patients were informed about the study aims and the need of their cooperation to minimize the effect of confounding factors upon the treatment outcome. Diagnosis of mild to moderate gingivitis

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was substantiated using current guidelines by a team of experienced clinical staff, double-blinded to the study outcome, as were the participants. Any patients presenting with any form of gingivitis (acute or chronic) that had progressed to periodontitis were excluded from the study. The diagnosis of gingivitis was based on clinical guidelines and included a detailed clinical history, signs and symptoms, and clinical (disclosing solution to disclose dental plaque)/radiographic examination [18]. Gingivitis, which is primarily caused by and/or associated with dental plaque, was graded on the extent and severity of bleeding on probing (BOP). This diagnostic system allows identifying incipient gingivitis even if few sites are affected. BOP scores higher than 30% were considered to indicate generalized gingivitis [18]. Patient allotment to a particular group was done by one of the researchers who had no role in patient treatment or evaluating the treatment outcome after intervention. Patients were assigned to groups by random sequencing generated using SPSS software for Windows, (version 21, Chicago, Illinois, USA). The 3 groups were defined on the basis of the intervention method (commercial mouthwash used) they received after routine treatment. The treatment included removal of plaque by using a combination of hand and ultrasonic scaling. A piezo electric ultrasonic scaler (Satelec Acteon Group, Bordeaux, France) using regular scaler tips was used with chairside distilled water cooling. Enamel polishing after scaling for all patients was done using a prophylaxis brush made of nylon bristles (Pro-Brush™, Kerr, Bioggio, Switzerland) and prophylaxis paste (Cleanic paste, Kerr, Bioggio, Switzerland) rotating at low speed. All patients were educated about the brushing method to be used (Bass method). All patients were provided with a medium-bristle toothbrush (Colgate-Palmolive Arabia, Ltd.) and a dentifrice (Colgate-Palmolive Arabia, Ltd.). The medium-bristled toothbrush has been found to be more effective for removing plaque in the posterior teeth, especially where the tooth surface is contoured toward interproximal areas. As part of the intervention, the patients also received a coded bottle that contained the mouthwash (group-based) and tablet provided by an operator who was blinded to the type of mouthwash in the bottle. Patients were instructed to use their mouthwash after brushing and refrain from drinking, eating, and mouth rinsing for at least 2 hours. The risk of confounding variables influencing the outcome of the study was thus controlled by randomization and restriction within treatment methodology.

Study groups comprised Group 1 patients who were blinded to use of 0.2% Chlorhexidine mouthwash [Chlorhexidine gluconate 0.2% in aqueous flavored base (11.6% alcohol, glycerin, sodium saccharin, flavoring agent, sorbitan diisostearate and water)] (Spimaco, Addwaeih, KSA), twice daily (morning/night) for 14 days; Group 2 patients were blinded to use of herbal mouthwash (Alpine Manuka Mouthwash, Nelson Honey, New Zealand) (Natural Manuka honey, Leptospermum scoparius leaf/flower, water, glycerin, oil – Mentha piperita, Triticum aestivum and Hordeum vulgare leaf extract, menthol, caramel, xylitol) twice daily (morning/night) for 14 days; and Group 3 patients blinded to use of PRO-Dental (Hyperbiotics, Probi, USA, Inc) [Lactobacillus reuteri, *Bifidobacterium bifidum* and *Lactobacillus rhamnosus* (0.1 billion colony-forming units [cfu]), zinc, lactilol and fructo oligosaccharides, polysorbate 20, sodium benzoate, oil (*Rosmarinus officinalis*, *Eugenia caryophyllus, Mentha viridis*), glycerin, xylitol, peppermint and water], 1 tablet dissolved in 10 ml of normal water and swished once in the morning for 14 days. The patients were instructed not to use any other aids for oral hygiene maintenance until the last follow-up.

Measures, Data Evaluation, Collection, and Analysis

Comparative evaluation in each group and between groups was performed after collecting the data for the following clinical parameters that were recorded at baseline and at 7, 14, and 28 days at regular follow-up. These included the following:

1. Plaque index (PI) (amount of visible dental plaque on lingual/vestibular tooth surfaces except the third molar [19]);
2. Gingival index (GI) (presence or absence of characteristics of inflammation such as edema, redness, swelling, and spontaneous bleeding) [20];
3. Modified Sulcular Bleeding index (MSBI) (presence or absence of bleeding upon gentle probing) [21].

At each follow-up visit, for each clinical parameter, inter-examiner reliability and intra-examiner reliability were ensured by evaluation by 2 different examiners and by repeated measurement of clinical parameter by the same assessor at 2 different times. The intra-class correlation coefficient calculated was 0.87, which was considered to be appropriate.

Statistical Analysis

Demographic characteristics were described in terms of frequency distribution (categorical variable), while the mean (continuous variable) and standard deviation (SD) were calculated for various clinical parameters. To assess homogeneity among the 3 groups, a chi-square test was used. The inter-group statistical comparison between means (continuous variable) was done using analysis of variance (ANOVA) with Bonferroni correction (to correct the experiment-wise error rate when using multiple *t* tests) for multiple-group comparisons. The intra-group statistical comparisons were done using ANOVA (repeated measures) in each study group. Assumption of normality (Kolmogorov-Smirnov test) was tested before subjecting each variable to ANOVA. For statistical comparisons, differences between study groups were considered to be statistically significant if the probable value was less than or equal to 0.05. Various levels of sensitivity for statistical significance were determined at multiple probable values (significant at $P<0.05$, very significant at $P<0.01$, and highly significant at $P<0.001$). Formulation of hypothesis

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Table 1. Demographic characteristics of study participants and their distribution status between the groups.

Parameter	Divisions	Group 1 (CHX) (n=15) n (%)	Group 2 (Manuka) (n=15) n (%)	Group 3 (ProD) (n=15) n (%)	Total	Chi square test P-value
Gender	Male	8 (53.3%)	9 (60.0%)	7 (46.7%)	24	χ^2=0.535 (NS) P=.76501
	Female	7 (46.7%)	6 (40.0%)	8 (53.3%)	21	
Age	21-25	3 (20.0%)	4 (26.7%)	5 (33.3%)	11	χ^2=0.5455 (NS) P=.9972
	26-30	4 (26.7%)	3 (20.0%)	4 (26.7%)	11	
	31-35	4 (26.7%)	4 (26.7%)	3 (20.0%)	12	
	36-40	4 (26.6%)	4 (26.6%)	3 (20.0%)	11	

CHX – chlorhexidine; ProD – prodental probiotic; n – number. Level of significance: NS (non-significant)=P≥0.05; * P<0.05.

was verified using alternatives (2-tailed) against every null hypothesis. The all-inclusive data were statistically investigated using SPSS for Windows, version 21 (Chicago, Illinois, USA).

Results

Frequency distributions (sex, age) as related to each group are presented in **Table 1**. The distribution of cases according to sex, studied across the 3 study groups, was a male-to-female sex ratio of 1.14: 1.00. There were no significant differences among the subjects in terms of sex or age, suggesting that the patients in the groups were homogeneously distributed in relation to the demographic characteristics, thus nullifying the statistical effect of confounding variables.

Inter-Group and Intra-Group Comparison of Mean Plaque Index (Table 2, Figure 2)

The mean scores of subjects in all the groups decreased from baseline to 28 days. Comparison of mean scores between groups at various time points showed that the difference in mean was very significant (*P*<0.01) between Group 1 and Group 3 (day 7), between Group 2 and Group 3 (days 14 and 28), whereas the difference in means was found to be highly significant (*P*<0.001) between Group 1 and Group 3 on day 28 (Table 2). Intra-group comparison (**Figure 2**) revealed a highly significant (P<0.001) difference in mean values at baseline compared to mean plaque index at days 7, 14, and 28 in all 3 experimental groups. The mean value at day 7 also differed significantly (*P*<0.05) compared to mean plaque index at day 14 in Groups 2 and 3.

Inter-Group and Intra-Group Comparison of Mean Gingival Index (Table 2, Figure 3)

The mean gingival index scores among all the groups were found to be higher at baseline, and declined among all groups by day 28, except in Groups 2 and 3, which both showed an increase from day 14 (Group 2 – 0.57±0.21 to 0.61±0.19; Group 3 – 0.85±0.16 to 0.89±0.14). When mean values for all groups were compared, the results show that the differences in mean between Groups 3 and 1 were highly significant (*P*<0.001) on days 7 and 28, while also being significant (*P*<0.05) on day 14 and that the differences in mean between Groups 3 and 2 were highly significant (*P*<0.001) at all observed time points (days 7, 14, 28). The intra-group comparison showed that the baseline values were highly significant (*P*<0.001) when compared to means on days 7, 14, and 28. Differences in mean values also were significant (*P*<0.05) in Groups 1 and 2 between days 7 and 28 and in Groups 2 and 3 between days 7 and 14 (**Figure 3**).

Inter-Group and Intra-Group Comparison of Mean Bleeding Index (Table 2, Figure 4)

The mean bleeding index scores among all the 3 groups were higher at baseline as compared to other time points. There was a decrease in the mean bleeding index scores from baseline to days 7 and 14, while an increase in mean bleeding index scores was observed on day 28 for all groups. When scores were compared between the 3 groups, the mean scores differed significantly (*P*<0.05) between Groups 1 and 3 on day 7, and the difference was very significant (*P*<0.01) between Groups 2 and 3 on days 7 and 28, and the difference was highly significant (*P*<0.001) on day 14. When comparisons were made within groups, the results showed that the difference in means between baseline values and the remaining 3 time points (days 7, 14, and 28) were highly significant (*P*<0.001), while there was no statistically significant difference in means between other groups (**Figure 4**).

Discussion

The present study was conducted to evaluate the clinically efficacy of 0.2% Chlorhexidine, Manuka (Alpine), and PRO-Dental

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Table 2. Comparative mean scores of clinical parameters among patients in various groups and the respective level of significance between various groups at different intervals of time.

Clinical parameters		Group 1 (CHX)	Group 2 (Manuka)	Group 3 (ProB)	P-value (Inter-Group)		
		Mean±SD	Mean±SD	Mean±SD	Group 1 vs group 2	Group 1 vs group 3	Group 2 vs group 3
Plaque Index	Baseline	1.35±0.23	1.43±0.21	1.31±0.16	0.883	0.999	0.356
	Day 7	0.73±0.14	0.79±0.15	0.92±0.16	0.840	0.004**	0.078
	Day 14	0.69±0.13	0.61±0.18	0.81±0.15	0.399	0.155	0.003**
	Day 28	0.66±0.11	0.71±0.15	0.87±0.13	0.999	0.001***	0.004**
Gingival Index	Baseline	1.35±0.12	1.39±0.19	1.41±0.23	0.999	0.999	0.999
	Day 7	0.75±0.15	0.76±0.10	0.98±0.15	0.999	0.001***	0.001***
	Day 14	0.66±0.20	0.57±0.21	0.85±0.16	0.562	0.031*	0.001***
	Day 28	0.61±0.16	0.61±0.19	0.89±0.14	0.999	0.001***	0.001***
Bleeding Index	Baseline	0.93±0.17	0.98±0.16	1.03±0.17	0.999	0.330	0.999
	Day 7	0.47±0.17	0.45±0.11	0.61±0.13	0.999	0.024*	0.008**
	Day 14	0.45±0.17	0.33±0.17	0.54±0.10	0.086	0.385	0.001***
	Day 28	0.46±0.15	0.38±0.19=7	0.55±0.10	0.411	0.324	0.009**

CHX – chlorhexidine; ProB – probiotics; vs – versus. Levels of significance: NS (not-significant)=P≥0.05; * Significant=P<0.05; ** Very significant=P<0.01; *** Highly significant=P<0.001.

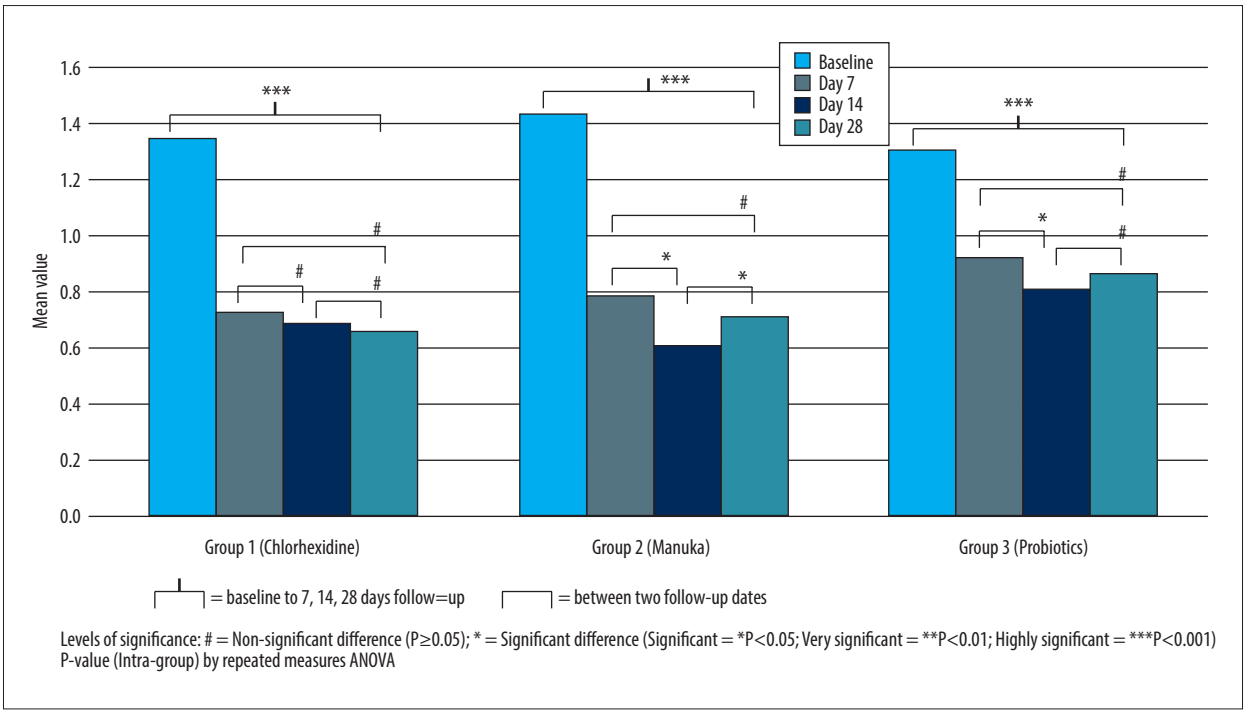

Figure 2. Intra-group comparison of mean plaque index scores between various groups at 4 different intervals of time. *Figure created using MS Excel, version 20H2 (OS build 19042,1466), windows 10 Pro, Microsoft corporation).*

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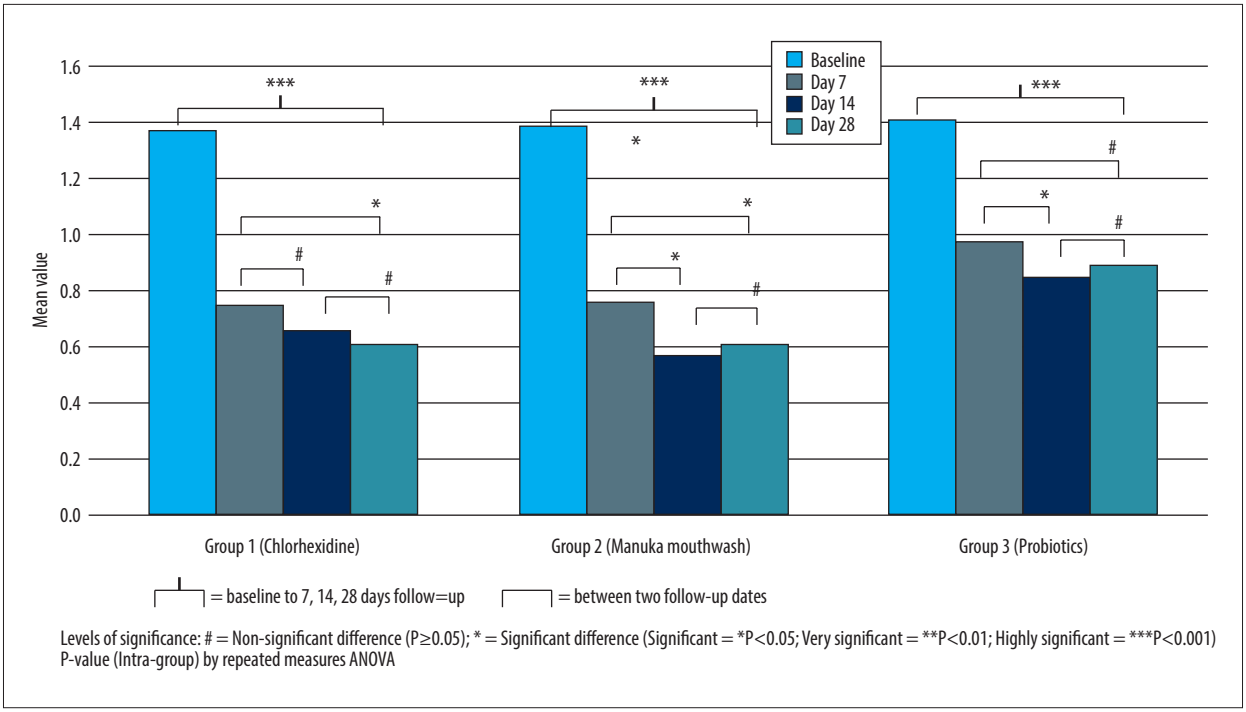

Figure 3. Intra-group comparison of mean gingival index scores between various groups at 4 different intervals of time. *Figure created using MS Excel, version 20H2 (OS build 19042,1466), Windows 10 Pro, Microsoft corporation).*

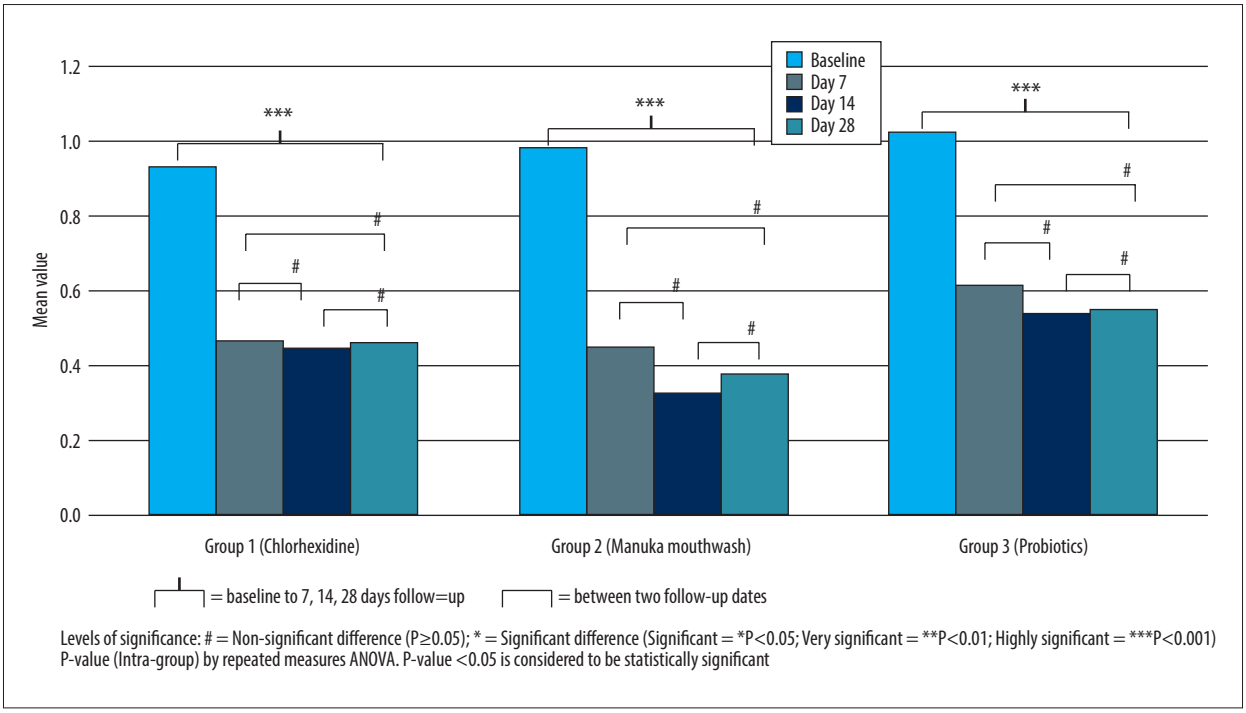

Figure 4. Intra-group comparison of mean bleeding index scores between various groups at 4 different intervals of time. *Figure created using MS Excel, version 20H2 (OS build 19042,1466), Windows 10 Pro, Microsoft corporation).*

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probiotic mouthwashes as an adjunct to mechanical therapy on clinical parameters such as PI, GI, and MSBI in patients with mild to moderate gingivitis. Chlorhexidine (CHX) is considered to be the criterion standard due to its proven efficiency and excellent sustained results [21,22]. Therefore, CHX is considered to be a positive control for comparison with other substances [23]. The results of this study showed that there was a significant decrease in mean PI from baseline to day 28 within the CHX group. This is due to the binding of CHX to tooth surfaces and oral mucosa for an extended period of time, causing reduction in both bacterial recolonization and disruption of the cytoplasmic membrane of the bacteria, being effective against both gram-positive and gram-negative bacteria. Also, the mean PI was lower in patients who used CHX mouthwash compared to patients who used Manuka and PRO-Dental formula probiotic mouthwash. A similar decrease in mean GI and mean bleeding index was also seen in the CHX group compared to other groups owing to decreased inflammation due to less plaque accumulation. Considering the adverse effects of CHX as long-term therapy, or in patients who cannot access certain parts of orthodontic appliances or prosthesis, its use has been limited or not actively recommended [24,25]. Thus, herbal mouthwashes like Manuka and biological agents like PRO-Dental formula have gained attention.

The overall results of this study show that the greatest decline in means from baseline to day 28 for PI (1.43 to 0.71), GI (1.39 to 0.61), and BI (0.98 to 0.33) was within Group 2 (Manuka). Clinically, it means that Manuka mouthwash brought higher improvements when measured on all 3 gingival health indices. The Manuka mouthwash contains herbs and essential oils besides containing antioxidants like ascorbic acid, phenolic acid, and flavonoids. The product comes from bees that are capable of pollinating *Leptospermum scoparius* (Manuka bush plant), growing predominantly in Australia and New Zealand. It has antibacterial activities while also being bacterial resistant and has been reported to act as a cariostatic agent active against Streptococcus and *Actinomyces naeslundii* [26], both of which are pioneers in dental plaque flora [24]. Reduction of dental plaque around the gingiva directly influences the rate and extent of gingival inflammation, which in turn reduces gingival bleeding. The anti-plaque effect is thought to be due to the bactericidal effect of hydrogen peroxide (oxidative damage to proteins, enzymes, and DNA) [27]. The direct effect on gingiva is also due to the antioxidants, which have a robust modulatory effect on gingival inflammation of diminishing the inflammation through reduction of oxidative stress and improving endothelial function. The decrease in gingival inflammation leads to decreases gingival bleeding. Therefore, Manuka mouthwash may be considered as a viable complement to anti-plaque and anti-gingivitis strategies, both short- and long-term [28]. The results in decreasing the plaque score by Manuka mouthwash as compared with baseline agree with results of Nayak et al [16] and Singhal et al [29]. However, in both studies Manuka was not as effective as CHX, which disagrees with the present study, perhaps due to the method of application of mouthwash. We used a patented commercial mouthwash, while Nayak et al used chewing lather made of honey and Singhal et al prepared a custom-made mouthwash (in their study, the lower effectiveness of honey mouthwash was associated with the heterogeneous nature of honey and interference by local oral factors). This suggests the proportion of ingredients in Manuka mouthwash plays a significant role in gingival health.

The results of our study showing the anti-gingivitis effect of Manuka mouthwash are in accordance with previous in vivo [30] and in vitro studies [31]. Reduction of *Porphyromonas gingivalis* in the plaque was observed in both studies. Jain et al reported that compared to CHX, there was more plaque reduction by honey and CHX with xylitol [32]. Our study did not find any difference between Group 1 and Group 2 for any clinical parameter at any time point. This suggests that Manuka mouthwash has the same properties as that of CHX, but since CHX is not recommended for long-term use, Manuka mouthwash is an alternative for long-term treatment maintenance.

We found a decrease in mean PI, GI, and bleeding index from baseline to day 28 in the probiotic group, but these was less worsening of clinical parameters compared to the CHX group and Manuka group. The probiotic mouthwash used in this study contains the strain *L. proxy* that can fight important oral pathogens, including Streptococcus mutants. Streptococcus mutants, apart from causing caries, also contribute to plaque formation by producing strong adhesive Dextrans that help bind to microorganisms on the tooth surface [33]. Probiotic mouthwash reduces plaque formation by inhibiting *Streptococcus mutans* and thereby improves overall gingival health [12]. The probiotic mouthwash also contains zinc, which is a powerful antioxidant and plays a vital role in scavenging free radicals, thereby reducing gingival inflammation and gingival bleeding [17,34]. The probiotics help in altering the balance of the oral flora toward the beneficial species and thereby reduce the bacterial mediated oral diseases such as gingivitis. There are various commercial preparations for probiotics, which include those that have Streptococcus species (Probiota) and those that contain Lactobacillus species. The one that contain Lactobacillus species has been observed to be more effective against *Porphyromonas gingivalis* [35], a species that is strongly associated with periodontitis caused by subgingival plaque. The results of this study agree with the results of Hanaa et al [36] and Vikas et al [37]. However, the former study was an in vitro study and used a Streptococcus-containing probiotic while the latter used Lactobacillus probiotic and was an in vivo study. The study using Streptococci probiotic did not find any significant changes in bacterial count after 30 days.

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Although patients of all the 3 groups experienced a decrease in mean PI, GI, and mean bleeding index from baseline to day 28, the reduction in clinical parameters was greater in the CHX group and Manuka group, with both groups showing no significant differences between them for any clinical parameter at any time point.

Study Strengths and Limitations

This study compared 3 commercial preparations, which eliminated influences that can result due to making a customized or a self-made mouthwash preparation. The results obtained are conclusive that Manuka mouthwash is a reliable alternative to CHX-containing mouthwash, which is generally not indicated when long-term chemical oral hygiene maintenance is desired, as in prosthodontic patients receiving full mouth rehabilitation or orthodontic patients undergoing fixed appliance correction of teeth. The study is limited by its small sample size, the sample chosen from a particular area, and all the limitations that are associated with a cross-sectional sample.

Conclusions

This study showed a decrease in mean PI, GI, and mean bleeding index from baseline to day 28 with the use of 0.2% Chlorhexidine, Manuka, and PRO-Dental formula probiotic mouthwash as adjunct to mechanical therapy in gingivitis patients, indicating that all the 3 mouthwashes are clinically effective in treating plaque-induced gingivitis. Patients using chlorhexidine and Manuka mouthwash experienced a substantial decrease in the clinical parameters among the 3 groups. To overcome the drawbacks of prescribing a CHX-based mouthwash for long-term maintenance of gingival and periodontal health, this study recommends using Manuka, which is equally effective to CHX, while minimizing the adverse effects (eg, staining, mucosal burning, and dehydration) associated with long-term use of CHX. However, long-term studies are needed to investigate the adverse effects of Manuka mouthwash.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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