

Article

Evaluation of a Toothpaste Containing Cetylpyridinium Chloride and Cymenol—A Randomized Clinical Trial

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Abstract: The primary objective was to assess the safety of a new toothpaste formulation containing cetylpyridinium chloride (CPC) and cymenol as active agents. In addition, clinical efficacy and patient perception were evaluated. A 6-week randomized clinical trial was designed and patients were randomly allocated to use a test or control dentifrice. Adverse effects were assessed at 3- and 6-week follow-up visits, together with the following clinical parameters: Turesky plaque index (PII), bleeding on marginal probing (BOMP), bleeding on probing (BOP) and Gründemann modification of the stain index (GMSI). Patient-reported outcome measures (PROMs) were also evaluated. A total of 60 patients were randomized and 55 were finally included in the analysis (test, 29; control, 26). Both toothpastes were safe and well tolerated by the patients. Statistically significant differences between test and control groups were observed for BOP changes between baseline week 6 ($p = 0.031$) and lower levels of plaque were registered in the test group at the 3- and 6-week visits ($p < 0.05$). No differences were observed for staining or PROMs. The use of a toothpaste containing CPC and cymenol was safe and not associated with any adverse effects. Significantly better clinical results were observed for the test group in terms of PII levels and BOP reductions.

Keywords: cetylpyridinium chloride; cymenol; toothpaste; tolerability; dental biofilm; gingival inflammation



Citation: Vasconcelos, V.; Laciari, F.; Matesanz, P.; Iniesta, M.; Alonso, B.; Roldán, S.; Serrano, J.; Furmanczyk, M.; Bustos, J.; Trullas, C.; et al. Evaluation of a Toothpaste Containing Cetylpyridinium Chloride and Cymenol—A Randomized Clinical Trial. *Appl. Sci.* **2024**, *14*, 105. <https://doi.org/10.3390/app14010105>

Academic Editor: Dorina Lauritano

Received: 1 December 2023

Revised: 18 December 2023

Accepted: 20 December 2023

Published: 21 December 2023



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1. Introduction

Oral biofilms are the main causative factor for caries, periodontal diseases, peri-implant diseases and oral halitosis. Hence, an adequate regular control of oral biofilms is critical not only to maintain oral health but also to prevent recurrent disease [1,2]. Therefore, since epidemiological and intervention studies have shown an association between periodontitis and the onset and progression of different systemic diseases, oral biofilm control is also important for maintaining overall health [3].

An efficient regular mechanical oral biofilm control is difficult to achieve and is limited by both subject-related factors such as poor motivation or education and lack of adequate compliance; and/or skills and tooth-related factors, such as the limited use of interdental cleaning devices and the presence of hard-to-reach areas. As a consequence, there is clear evidence [4,5] that for some patients, mechanical biofilm control is not sufficient for preventing or controlling oral diseases. In these patients, the adjuvant use of chemical

agents with antimicrobial effects, as mouthwashes or toothpastes, has shown to be useful in dental biofilm control and maintenance of oral/periodontal health [6]. Furthermore, a recent published systematic review showed that antiseptic therapies used adjunctively to mechanical plaque control were equally effective in reducing gingivitis and plaque in patients with gingivitis on intact periodontium or in previously treated periodontitis patients with gingival inflammation [7].

Toothpaste, by combining the antimicrobial effect provided by its active ingredients, with the mechanical effect of toothbrushing, is the preferred method for controlling oral biofilms by both dentists and patients [6], and consequently, is the most widespread population-based oral hygiene method.

Although chlorhexidine (CHX) is currently considered as the most effective and long-lasting antiplaque agent available [6], its widespread use is limited due to its side effects with prolonged use, including tooth and tongue pigmentation, taste alteration, mucous membrane irritation and hypersensitivity reactions [8]. Moreover, due to its high cationic nature, CHX-based toothpastes are difficult to formulate since other anionic substances, such as surfactants, may reduce its bioavailability and, consequently, its efficacy [6].

As an alternative, the use of cetylpyridinium chloride (CPC)-based toothpastes has been suggested for oral biofilm control [6]. The antimicrobial activity of CPC has been studied for more than 70 years with proven antimicrobial effect against Gram-positive bacteria, fungi and yeasts [9]. CPC-containing products are considered as safe and effective for the treatment of plaque-induced gingivitis by the Food and Drug Administration (FDA) [10]. Its efficacy, however, may depend on the specific formulation, due to its astringent characteristics and the possible interactions with excipients or other components. Therefore, it is not uncommon to find two CPC products formulated with the same concentration, but with significantly different substantivity, bioavailability and ability to penetrate within the biofilm, which may significantly impact its efficacy [11]. To overcome some of these limitations, other substances with biological activity synergistic with CPC have been added to oral hygiene products [12]. In fact, the FDA has recognized the effectivity of natural products such as plant extracts and the essential oils of eucalyptus, menthol, methyl salicylate and thymol (cymenol) in mouth rinses and toothpastes due to their antibacterial and antibiofilm properties. Kakar et al. [13] confirmed that the use of a 0.1% cymenol-containing toothpaste over a 12-week period provides a statistically significant benefit maintaining gingival health as compared to a sodium fluoride control toothpaste. Moreover, a recent study concluded that CPC and cymenol formulated in mouth rinses may act synergistically, extending the substantivity of their action [14].

However, there is limited information in the literature on the adjunctive efficacy of CPC-based toothpastes and even more scarce, about the effects of toothpastes formulated with CPC combined with other antimicrobial agents. Therefore, the objective of this clinical trial was to evaluate the safety and tolerability of a new toothpaste formulated with CPC and cymenol, and secondarily, its clinical efficacy and patient perception, when compared to a negative control, a fluoride-based toothpaste available on the market.

2. Materials and Methods

2.1. Study Design

This randomized controlled clinical trial was designed as a single center, double-blind pilot study.

2.2. Ethical Aspects

This study complies with the ethical principles of the Declaration of Helsinki (Edinburgh revision of October 2000), with the Standards of Good Clinical Practice and with the Spanish Code of Ethics. The protocol was registered at the ISRCTN registry (Springer Nature code ISRCTN17497809 (<https://doi.org/10.1186/ISRCTN17497809>, accessed on 1 November 2023) and approved by the Ethics Committee of the Hospital Clínico San Carlos,

Madrid (C.I.21/262-EC_X). Informed consent was obtained from all subjects involved in the study.

2.3. Study Population

The study was conducted at the Postgraduate Periodontal Clinic at the Faculty of Dentistry in the Complutense University of Madrid, Spain. Consecutive subjects diagnosed with gingivitis or previously treated periodontitis patients with gingival inflammation, according to the 2018 World Workshop Classification [15], were selected if fulfilling the following inclusion and exclusion criteria.

2.3.1. Inclusion Criteria

- Over 18 years of age;
- Systemically healthy, defined according to the criteria of the American Society of Anesthesiologists (ASA) [16], as ASA type I or II (see also exclusion criteria);
- Presence of at least three evaluable teeth in each quadrant;
- Moderate gingival inflammation ($\geq 40\%$ bleeding on marginal probing, BOMP) [17] and Turesky plaque index ≥ 1.5 [18];
- Absence of probing depths (PD) ≥ 5 mm;
- No fixed orthodontic treatments or removable prostheses;
- Brushed their teeth regularly (at least twice a day).

2.3.2. Exclusion Criteria

- Untreated or uncontrolled periodontitis;
- Regular users of mouthwashes during the month prior to the screening;
- Antibiotic intake within the previous month;
- Pregnant women;
- Any chronic disease or medication that may influence gingival inflammation;
- Conditions requiring antibiotic coverage.

2.4. Study Visits and Intervention

The study had a duration of 6 weeks and consisted of four visits.

2.4.1. Screening Visit

The screening visit assessed whether the subjects met the inclusion/exclusion criteria.

2.4.2. Baseline Visit

Clinical parameters were recorded on all teeth, except the third molars. After the clinical examination, subjects were randomly assigned to one of the two groups: the test toothpaste (Bexident[®] Encías Uso Diario, ISDIN, Barcelona, Spain), containing CPC and cymenol as active ingredients; and the control toothpaste (Colgate Protection Caries toothpaste, Colgate-Palmolive España S.A., Madrid, Spain), containing fluoride and sodium monofluorophosphate as active ingredients. No further treatment was provided during the study period. Participants received standardized verbal and written instructions asking them to brush three times per day, for two minutes, after breakfast, lunch and dinner, with a provided manual toothbrush (UltraThin ProGumCare[®], OralB, Madrid, Spain) and the assigned toothpaste, and were asked to fill out the provided compliance forms, as well as any comments or adverse effects experienced during the study period. All subjects were blinded to their product assignment and received their product kits, which included the manual toothbrush and the appropriate amount of the randomly assigned toothpaste in a white tube (coded A or B for the study coordinator, and with their unique identification code for the patient). No further toothbrushing or interdental cleaning instructions were provided.

2.4.3. Week 3 and Week 6 Visits

Clinical parameters were recorded during the visit after 3 and 6 weeks of product use. Subjects were asked about the occurrence of adverse effects and their perceptions (PROMs) on the use of the assigned toothpaste by filling a questionnaire. Finally, the study coordinator collected empty and unused toothpaste tubes. At the end of the 6-week visit, all subjects received professional mechanical plaque removal (PMPR).

2.5. Randomization and Blinding

Subjects were identified through a unique trial number. Participants were randomly allocated to one of the two treatment groups (test or control toothpaste). Randomization was performed, by a researcher not involved in the clinical evaluations, using random numbers from a computer-generated list with Microsoft Excel® software (version 16.78.3), in blocks of six patients. All subjects were blinded to their product assignment, as well as all researchers involved in patient recruitment or evaluation.

2.6. Primary Outcome-Safety and Tolerability

Each participant was interviewed regarding adverse events at each visit. Visual soft- and hard-tissue examinations of the oral cavity were performed at every visit to assess the safety of the products. Patients' reports of adverse events were also recorded.

2.7. Secondary Outcomes

2.7.1. Compliance

Compliance with the assigned products was assessed by the information provided by the patients at every appointment and by the remaining toothpaste returned at week 6.

2.7.2. Patient Reported Outcome Measures (PROMs)

All participants filled out a predefined questionnaire after using their assigned products (at 3–6 weeks).

2.7.3. Clinical Outcome Variables

Two trained and calibrated researchers (V.V. and F.L.) blinded to the treatment assignment performed all the examinations on 30 patients each. Intra- and inter-examiner calibration was carried out through double measurement sessions of selected clinical parameters (listed in the next paragraph) in four patients. The agreement between examiners and within the same examiner was determined using kappa coefficients. The intra- and inter-examiner agreement ranged from 0.72 to 0.90 kappa scores [19].

Clinical examinations were performed in the following order:

- The Gründemann modification of the stain index (GMSI) [20], modified by Koertge and Gunsolley [21], in the upper and lower anterior teeth, buccal sites, by evaluating standardized clinical photographs;
- The plaque index (PII) of Quigley and Hein [22], modified by Turesky et al. [18], was assessed at six areas per tooth, using a plaque-disclosing solution (Plac-Control®, Dentaïd, Barcelona, Spain);
- Bleeding on marginal probing (BOMP) [17,23], recording the presence or absence of bleeding within 30 s of probing on a 0–2 scale;
- Bleeding on probing (BOP) [24] by dichotomous assessment of bleeding after gentle probing.

In addition, a careful inspection of the gingival tissues was carried out to identify possible lesions in the gingival margin or adjacent mucosa. The presence of these lesions was recorded, together with their location.

2.8. Data Analysis

2.8.1. Sample Size Calculation

A convenience sample size of 30 patients per group was established as this RCT was considered as a pilot study. A post hoc power calculation was conducted using Macro!NSize [25], for clinical outcomes (PII and BOP) in the changes from baseline to 6 weeks, using a two-tailed test with an alpha of 0.05%. Based on the observed effect size, the present sample size of 55 participants provided a power of 42% for PII and 71.4% for BOP, to detect statistically significant differences between groups.

2.8.2. Statistical Analysis

Data were expressed as means and standard deviation (SD), and as median and interquartile ranges (IQR) for non-parametric data. Categorical data were expressed as percentages. Data on categorical variables were compared using the chi-square or Fisher's exact tests. The Shapiro–Wilk goodness of fit test was used to determine the normal distribution of quantitative variables. The differences in quantitative variables inter- and intragroup at baseline, week 3 and week 6, were determined by repeated measures ANOVA for parametric data and Friedman test for non-parametric data, both with Bonferroni correction. The change variables between baseline and weeks 3 and 6, were examined by the Student's *t*-test or the Mann–Whitney U tests. The average of all the responses for each of the PROM items was calculated also taking into account those responses from the subjects excluded from the analysis due to their inability to attend any of the visits.

The results were considered statistically significant if $p < 0.05$. All analyses were performed with IBM® SPSS® STATISTICS 27.0 (IBM Corporation, Armonk, NY, USA). For all variables, the patient was the statistical unit for analysis.

3. Results

3.1. Demographic Data

The study took place from January to December 2022. A total of 110 patients were screened until the required 60 patients were reached (see Figure 1). After the baseline visit, two patients of the control group had to be excluded because they took medications that could affect the results. Additionally, three patients (two from the control group and one from the test group) were unable to attend the week 3 or week 6 visits due to work-related reasons. The final distribution of patients is presented in Table 1. No statistically significant differences were found between the groups at the baseline visit for any of the clinical outcomes evaluated. In the test group, the sample consisted of 11 males and 18 females, ranging from 21 to 72 years of age, with a mean age of 39.24 (standard deviation, SD = 17.77 years), with three patients being current smokers. The control group consisted of 12 males and 14 females, ranging from 21 to 74 years, with a mean age of 43.23 (SD = 18.01 years), and two of the patients being current smokers.

Table 1. Demographic characteristics of the complete patient sample and by study group, analyzed by means of different statistical tests (see column “*p* value”).

		All	Test Group	Control Group	<i>p</i>
n		55	29	26	
Age	Mean (SD)	41.13 (0.17)	39.24 (17.77)	43.23 (18.01)	Mann–Whitney
	Min–Max	21–74	21–72	21–74	0.438
Gender	Male [n (%)]	23 (41.8)	11 (37.9)	12 (46.2)	Chi-square
	Female [n (%)]	32 (58.2)	18 (62.1)	14 (53.8)	0.537
Smoking	No [n (%)]	50 (90.9)	26 (89.7)	24 (92.3)	Fisher exact
	Yes [n (%)]	5 (9.1)	3 (10.3)	2 (7.7)	1.000

Table 1. Cont.

		All	Test Group	Control Group	<i>p</i>
Systemic conditions	No [n (%)]	47 (85.5)	23 (79.3)	24 (92.3)	Fisher exact
	Yes [n (%)]	8 (14.5)	6 (20.7)	2 (7.7)	0.257
Chronic medications	No [n (%)]	35 (63.6)	18 (62.1)	17 (65.4)	Chi-square
	Yes [n (%)]	20 (36.4)	11 (37.9)	9 (34.6)	0.799
Allergies	No [n (%)]	50 (90.9)	26 (89.7)	24 (92.3)	Fisher exact
	Yes [n (%)]	5 (9.1)	3 (10.3)	2 (7.7)	1.000
Stress	No [n (%)]	35 (63.6)	20 (69.0)	15 (57.7)	Chi-square
	Yes [n (%)]	20 (36.4)	9 (31.0)	11 (42.3)	0.386

n: sample size; SD: standard deviation.

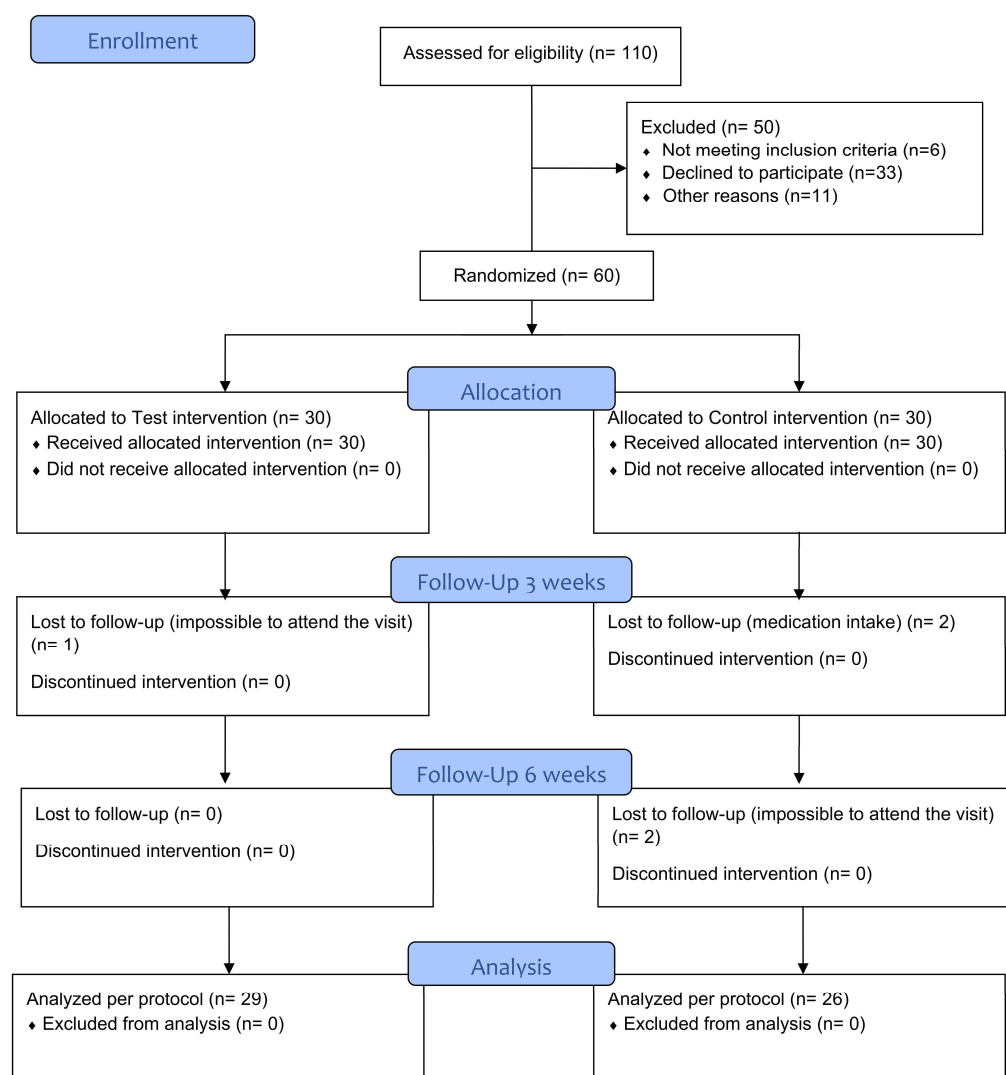


Figure 1. CONSORT flow diagram (version 2010) of the clinical trial.

3.2. Primary Outcome-Safety and Tolerability

The products were safe and well tolerated by the subjects, and no one reported any side effects or adverse symptoms.

3.3. Patient Compliance and PROMs

Both groups demonstrated a similar degree of compliance, as measured by the amount of remaining toothpaste in the returned tubes (Table 2), with a tendency for a higher consumption of toothpaste in the control group for the 3–6 week period ($p = 0.059$).

Table 2. Remaining amount of toothpaste, in milligrams, after each follow-up visit, analyzed with Student's *t*-test.

	Test Group	Control Group	Mean Difference	95% CI		<i>p</i>
	Mean (SD)	Mean (SD)		Lower Bound	Upper Bound	
n	28	22				
3 weeks	59.67 (33.35)	61.12 (38.70)	−1.456	−21.96	19.04	0.887
6 weeks	61.67 (35.51)	41.48 (38.20)	20.192	−0.83	41.22	0.059
Total	122.10 (41.45)	107.19 (51.10)	14.915	−11.39	41.22	0.260

n: sample size; SD: standard deviation; CI: confidence interval.

The results did not reveal any statistically significant difference between treatment groups in terms of the subject's appreciation of the assigned toothpaste in terms of consistency, aroma, taste, amount of foam, cleanness feeling, healthier mouth, fresher mouth and breath, less swollen and bleeding gums, “stronger gum” and “stronger tooth” feeling (Table 3). However, patients in the test group mentioned that they felt that the toothpaste removed less plaque than normal, which did not occur in the control group ($p = 0.017$). Both groups indicated that they would buy and recommend the assigned product.

Table 3. Mean, standard deviation (SD), median and interquartile range (IQR) for the scores of the toothpaste evaluation items (patient reported outcome measures, PROMs). The analysis is based on the average of the responses to the 3-week and 6-week questionnaires and by means of Mann–Whitney U tests. Answers were given on a scale of 1 to 4, except for the final evaluation of the product (1–10).

	Test Group			Control Group			<i>p</i>
	n	Mean (SD)	Median (IQR)	n	Mean (SD)	Median (IQR)	
Consistency	30	3.66 (0.37)	3.75 (0.50)	30	3.70 (0.38)	4.00 (0.50)	0.683
Aroma	30	3.50 (0.45)	3.50 (1.00)	30	3.56 (0.44)	3.50 (1.00)	0.581
Taste	30	3.45 (0.57)	3.50 (1.00)	30	3.60 (0.48)	4.00 (1.00)	0.324
Amount of foam	30	3.50 (0.55)	3.50 (1.00)	30	3.51 (0.59)	3.75 (1.00)	0.794
Freshness	30	3.05 (0.72)	3.00 (0.50)	30	3.26 (0.61)	3.25 (1.00)	0.278
Cleanness feeling	30	3.26 (0.66)	3.50 (0.63)	30	3.38 (0.55)	3.25 (1.00)	0.672
Healthier teeth feeling	30	3.03 (0.66)	3.00 (0.50)	30	3.28 (0.55)	3.25 (0.63)	0.119
Fresh mouth	30	3.18 (0.66)	3.00 (0.63)	30	3.36 (0.61)	3.50 (1.00)	0.264
Fresh breath	30	3.08 (0.67)	3.00 (0.63)	30	3.25 (0.65)	3.25 (1.00)	0.278
Less swollen gums	30	3.00 (0.57)	3.00 (1.00)	30	3.11 (0.72)	3.00 (1.13)	0.339
Reduced gum bleeding	30	3.21 (0.61)	3.00 (0.63)	29	3.15 (0.75)	3.00 (1.00)	0.900
Lasting reduction of bleeding and swelling	30	3.01 (0.71)	3.00 (1.00)	28	3.12 (0.66)	3.00 (1.38)	0.671
Healthy mouth feeling	30	3.21 (0.58)	3.25 (0.50)	30	3.40 (0.59)	3.50 (1.00)	0.217
Gentle on gums	30	3.61 (0.42)	3.50 (0.50)	30	3.60 (0.44)	3.75 (1.00)	0.905
Sensitive gum care	30	3.46 (0.57)	3.50 (1.00)	30	3.43 (0.52)	3.50 (1.00)	0.664

Table 3. Cont.

	Test Group			Control Group			<i>p</i>
	<i>n</i>	Mean (SD)	Median (IQR)	<i>n</i>	Mean (SD)	Median (IQR)	
Removes dental plaque	30	2.98 (0.63)	3.00 (0.63)	29	3.34 (0.58)	3.00 (1.00)	0.017
Stronger teeth feeling	30	3.03 (0.49)	3.00 (0.13)	30	3.15 (0.60)	3.00 (0.50)	0.321
Stronger gum feeling	30	3.18 (0.56)	3.00 (0.50)	29	3.10 (0.71)	3.00 (0.50)	0.911
Gum relief feeling	30	3.18 (0.51)	3.00 (0.50)	30	3.30 (0.61)	3.00 (1.00)	0.437
Overall rating	30	3.05 (0.59)	3.00 (0.50)	30	3.33 (0.46)	3.00 (1.00)	0.077
Buy the product	30	3.05 (0.68)	3.00 (0.63)	30	3.21 (0.50)	3.00 (0.50)	0.371
Recommend the product	30	3.15 (0.61)	3.00 (0.63)	30	3.20 (0.50)	3.00 (0.50)	0.760
Product evaluation	19	7.65 (1.10)	8.00 (1.00)	18	8.00 (1.28)	8.00 (2.00)	0.558

n: sample size; SD: standard deviation; IQR: interquartile range; *p* values in bold represent statistically significant differences.

3.4. Clinical Outcomes Variables

At the baseline visit, no statistically significant differences were found between groups for any clinical outcome variable, including PII, BOMP, BOP and GMSI.

3.4.1. Plaque Index (PII)

A greater overall PII reduction was noted in the test group as compared to the control group, after 3 (0.28 vs. 0.13, respectively) and 6 weeks (0.33 vs. 0.14, respectively), with statistically significant intergroup differences at week 3 (2.43 vs. 2.65, respectively, $p = 0.028$) and at week 6 (2.38 vs. 2.64, respectively, $p = 0.011$) (Table 4). The same was true for the lingual and proximal areas. Additionally, intragroup analyses in the test group showed statistically significant PII decreases between baseline and week 3, and baseline and week 6 (Table 4), including buccal, lingual and proximal areas.

Table 4. Plaque index (PII) analyzed by means of repeated measures ANOVA and Student's *t*-test.

	Test Group	Control Group	Mean Difference	95% CI Bounds		<i>p</i>
	Mean (SD)	Mean (SD)		Lower	Upper	
n	29	26				
All sites						
Baseline	2.72 (0.40) *	2.78 (0.36)	−0.069	−0.27	0.14	0.514
3 weeks	2.43 (0.36)	2.65 (0.33)	−0.215	−0.40	−0.02	0.028
6 weeks	2.38 (0.43) **	2.64 (0.28)	−0.265	−0.46	−0.06	0.011
Change BL-3w	0.28 (0.37)	0.13 (0.39)	0.146	−0.06	0.35	0.167
Change BL-6w	0.33 (0.44)	0.14 (0.40)	0.195	−0.03	0.42	0.095
Buccal						
Baseline	2.70 (0.52) *	2.68 (0.45)	0.013	−0.25	0.28	0.921
3 weeks	2.44 (0.47)	2.52 (0.43)	−0.078	−0.32	0.16	0.530
6 weeks	2.39 (0.49) **	2.53 (0.38)	−0.142	−0.38	0.09	0.243
Change BL-3w	0.25 (0.43)	0.16 (0.50)	0.091	−0.16	0.34	0.477
Change BL-6w	0.30 (0.55)	0.15 (0.46)	0.154	−0.12	0.43	0.270

Table 4. Cont.

	Test Group	Control Group	Mean Difference	95% CI Bounds		<i>p</i>
	Mean (SD)	Mean (SD)		Lower	Upper	
Lingual						
Baseline	2.74 (0.49) *	2.89 (0.41)	−0.151	−0.39	0.09	0.225
3 weeks	2.43 (0.40)	2.78 (0.36)	−0.353	−0.56	−0.14	0.001
6 weeks	2.37 (0.50) **	2.76 (0.32)	−0.389	−0.62	−0.15	0.002
Change BL-3w	0.30 (0.43)	0.10 (0.38)	0.201	−0.02	0.42	0.076
Change BL-6w	0.36 (0.46)	0.12 (0.41)	0.237	−0.00	0.47	0.051
Proximal						
Baseline	2.86 (0.40) *	2.93 (0.39)	−0.063	−0.28	0.15	0.564
3 weeks	2.58 (0.38)	2.80 (0.34)	−0.222	−0.42	−0.02	0.030
6 weeks	2.51 (0.46) **	2.80 (0.26)	−0.284	−0.49	−0.07	0.008
Change BL-3w	0.28 (0.39)	0.12 (0.38)	0.137	−0.05	0.37	0.137
Change BL-6w	0.34 (0.45)	0.12 (0.41)	0.220	−0.01	0.45	0.067

n: sample size; SD: standard deviation; CI: confidence interval; BL: baseline; 3w: 3 weeks; 6w: 6 weeks; */**: statistically significant ($p < 0.05$) intragroup differences BL-3w* or BL-6w**. *p* values in bold represent statistically significant ($p < 0.05$) intergroup differences.

3.4.2. Bleeding on Marginal Probing Index (BOMP)

No statistically significant differences were found between groups in terms of BOMP at any visit (Table 5), except for a significant reduction in the test group for the lingual area between baseline and week 6 ($p = 0.049$). Intragroup assessments revealed that both groups demonstrated significant reductions between baseline and week 3 and week 6 in the overall, buccal and proximal areas (Table 5). In lingual areas, the control group did not show significant differences at week 6 ($p = 0.133$) and the test group showed significant differences at week 3 ($p < 0.001$) and at week 6 ($p = 0.000$).

Table 5. Bleeding on marginal probing index (BOMP) analyzed by means of Mann–Whitney U test and Friedman test for paired samples with Bonferroni correction.

	Test Group		Control Group		<i>p</i>
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
n	29	29	26	26	
All sites					
Baseline	0.29 (0.17)	0.25 (0.25) *	0.31 (0.18)	0.29 (0.18) *	0.913
3 weeks	0.18 (0.15)	0.13 (0.13)	0.20 (0.16)	0.19 (0.15)	0.315
6 weeks	0.14 (0.12)	0.12 (0.15) **	0.21 (0.16)	0.17 (0.15) **	0.085
Change BL-3w	0.11 (0.14)	0.09 (0.17)	0.10 (0.13)	0.07 (0.19)	0.418
Change BL-6w	0.15 (0.11)	0.11 (0.19)	0.10 (0.14)	0.08 (0.17)	0.189
Buccal					
Baseline	0.24 (0.19)	0.19 (0.24) *	0.24 (0.16)	0.19 (0.12) *	0.698
3 weeks	0.14 (0.15)	0.08 (0.15)	0.14 (0.11)	0.11 (0.11)	0.375
6 weeks	0.10 (0.12)	0.06 (0.16) **	0.13 (0.13)	0.08 (0.12) **	0.174
Change BL-3w	0.10 (0.17)	0.08 (0.16)	0.10 (0.13)	0.10 (0.17)	0.813
Change BL-6w	0.14 (0.12)	0.09 (0.19)	0.11 (0.15)	0.11 (0.14)	0.680

Table 5. Cont.

	Test Group		Control Group		<i>p</i>
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Lingual					
Baseline	0.35 (0.18)	0.31 (0.23) *	0.38 (0.25)	0.36 (0.29) *	0.704
3 weeks	0.22 (0.18)	0.17 (0.17)	0.27 (0.23)	0.20 (0.21)	0.362
6 weeks	0.19 (0.15)	0.15 (0.17) **	0.28 (0.23)	0.21 (0.27)	0.049
Change BL-3w	0.12 (0.17)	0.13 (0.17)	0.11 (0.18)	0.05 (0.25)	0.292
Change BL-6w	0.16 (0.15)	0.14 (0.21)	0.09 (0.16)	0.08 (0.28)	0.159
Proximal					
Baseline	0.36 (0.21)	0.31 (0.33) *	0.37 (0.27)	0.29 (0.25) *	0.906
3 weeks	0.22 (0.22)	0.14 (0.15)	0.24 (0.21)	0.20 (0.21)	0.197
6 weeks	0.18 (0.18)	0.12 (0.24) **	0.25 (0.22)	0.21 (0.22) **	0.093
Change BL-3w	0.14 (0.20)	0.12 (0.19)	0.12 (0.17)	0.11 (0.26)	0.433
Change BL-6w	0.18 (0.15)	0.14 (0.26)	0.11 (0.17)	0.11 (0.22)	0.152

n: sample size; SD: standard deviation; IQR: interquartile range; BL: baseline; 3w: 3 weeks; 6w: 6 weeks; */**: statistically significant ($p < 0.05$) intragroup differences BL-3w* or BL-6w**. *p* values in bold represent statistically significant ($p < 0.05$) intergroup differences.

3.4.3. Bleeding on Probing (BOP)

Statistically significant intergroup differences were detected in the changes between baseline and week 6 for the overall (reduction of 0.19 in the test group and of 0.12 in the control group, $p = 0.031$) and buccal areas (reductions of 0.16 and 0.07, respectively, $p = 0.028$) (Table 6). No other statistically significant differences were found between the groups at any visit, or in the changes from baseline to week 3. In terms of intragroup assessments (Table 6), both groups demonstrated significant reductions after 3 and 6 weeks, in all areas.

Table 6. Bleeding on probing (BOP) analyzed by means of repeated measures ANOVA and Student's *t*-test.

	Test Group	Control Group	Mean Difference	95% CI Bounds		<i>p</i>
	Mean (SD)	Mean (SD)		Lower	Upper	
n	29	26				
All sites						
Baseline	0.46 (0.13) *	0.42 (0.16) *	0.045	−0.03	0.12	0.274
3 weeks	0.29 (0.13)	0.28 (0.19)	0.002	−0.08	0.09	0.961
6 weeks	0.26 (0.13) **	0.30 (0.14) **	−0.031	−0.10	0.04	0.412
Change BL-3w	0.17 (0.14)	0.13 (0.11)	0.042	−0.02	0.11	0.238
Change BL-6w	0.19 (0.13)	0.12 (0.10)	0.075	0.00	0.14	0.031
Buccal						
Baseline	0.36 (0.16) *	0.31 (0.18) *	0.050	−0.04	0.14	0.287
3 weeks	0.21 (0.13)	0.22 (0.15)	−0.007	−0.08	0.07	0.857
6 weeks	0.20 (0.12) **	0.23 (0.12) **	−0.037	−0.10	0.03	0.290
Change BL-3w	0.15 (0.14)	0.09 (0.14)	0.057	−0.02	0.13	0.148
Change BL-6w	0.16 (0.12)	0.07 (0.16)	0.087	0.00	0.16	0.028

Table 6. Cont.

	Test Group	Control Group	Mean Difference	95% CI Bounds		<i>p</i>
	Mean (SD)	Mean (SD)		Lower	Upper	
Lingual						
Baseline	0.56 (0.15) *	0.52 (0.17) *	0.039	−0.05	0.12	0.395
3 weeks	0.36 (0.18)	0.35 (0.24)	0.013	−0.10	0.13	0.819
6 weeks	0.32 (0.17) **	0.35 (0.20) **	−0.023	−0.12	0.07	0.649
Change BL-3w	0.19 (0.17)	0.17 (0.13)	0.025	−0.06	0.11	0.555
Change BL-6w	0.22 (0.19)	0.16 (0.11)	0.062	−0.02	0.14	0.147
Proximal						
Baseline	0.53 (0.14) *	0.50 (0.15) *	0.035	−0.04	0.11	0.403
3 weeks	0.33 (0.16)	0.33 (0.21)	0.000	−0.10	0.10	0.996
6 weeks	0.31 (0.17) **	0.35 (0.15) **	−0.042	−0.13	0.04	0.360
Change BL-3w	0.19 (0.17)	0.16 (0.14)	0.034	−0.05	0.12	0.433
Change BL-6w	0.22 (0.15)	0.14 (0.12)	0.076	−0.00	0.15	0.051

n: sample size; SD: standard deviation; CI: confidence interval; BL: baseline; 3w: 3 weeks; 6w: 6 weeks; */**: statistically significant ($p < 0.05$) intragroup differences BL-3w* or BL-6w**. *p* values in bold represent statistically significant ($p < 0.05$) intergroup differences.

3.4.4. Gründemann Modification of the Stain Index (GMSI)

As shown in Table 7, no statistically significant differences were observed at baseline, week 3 and week 6 visits between groups. The same results were found when comparing the intragroup assessment for both groups; no statistically significant differences were detected (Table 7).

Table 7. Gründemann modification of the stain index (GMSI) analyzed by means of Mann–Whitney U test and Friedman test for paired samples with Bonferroni correction.

	Test Group		Control Group		<i>p</i>
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
n	29	29	26	26	
All					
Baseline	0.38 (0.27)	0.41 (0.35)	0.39 (0.26)	0.40 (0.26)	0.960
3 weeks	0.38 (0.25)	0.42 (0.30)	0.39 (0.26)	0.40 (0.31)	0.807
6 weeks	0.39 (0.25)	0.42 (0.30)	0.41 (0.28)	0.40 (0.33)	0.807
Change BL-3w	0.00 (0.08)	0.00 (0.01)	−0.00 (0.05)	0.00 (0.01)	0.792
Change BL-6w	−0.00 (0.09)	0.00 (0.01)	−0.02 (0.06)	0.00 (0.03)	0.892
Marginal					
Baseline	0.36 (0.27)	0.41 (0.40)	0.34 (0.27)	0.33 (0.36)	0.691
3 weeks	0.34 (0.24)	0.33 (0.40)	0.36 (0.30)	0.33 (0.40)	0.980
6 weeks	0.35 (0.24)	0.33 (0.39)	0.39 (0.32)	0.36 (0.47)	0.926
Change BL-3w	0.02 (0.12)	0.00 (0.01)	−0.01 (0.09)	0.00 (0.01)	0.640
Change BL-6w	0.01 (0.12)	0.00 (0.01)	−0.04 (0.12)	−0.00 (0.08)	0.057

Table 7. Cont.

	Test Group		Control Group		<i>p</i>
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Incisal					
Baseline	0.40 (0.31)	0.50 (0.46)	0.43 (0.27)	0.45 (0.16)	0.818
3 weeks	0.42 (0.30)	0.50 (0.41)	0.42 (0.26)	0.49 (0.21)	0.959
6 weeks	0.43 (0.30)	0.50 (0.32)	0.43 (0.27)	0.48 (0.21)	0.959
Change BL-3w	−0.01 (0.09)	0.00 (0.00)	0.00 (0.04)	0.00 (0.00)	0.658
Change BL-6w	−0.02 (0.10)	0.00 (0.00)	0.00 (0.03)	0.00 (0.00)	0.917
Proximal					
Baseline	0.63 (0.48)	0.75 (0.96)	0.67 (0.38)	0.75 (0.45)	0.986
3 weeks	0.63 (0.46)	0.67 (0.84)	0.67 (0.39)	0.74 (0.48)	0.728
6 weeks	0.64 (0.45)	0.73 (0.84)	0.69 (0.41)	0.74 (0.45)	0.741
Change BL-3w	−0.00 (0.13)	0.00 (0.01)	0.00 (0.05)	0.00 (0.01)	0.986
Change BL-6w	−0.00 (0.13)	0.00 (0.00)	−0.02 (0.07)	−0.00 (0.06)	0.075

n: sample size; SD: standard deviation; IQR: interquartile range; BL: baseline; 3w: 3 weeks; 6w: 6 weeks.

4. Discussion

The present study demonstrates that the adjuvant use of a toothpaste with cetylpyridinium chloride and cymenol was safe and tolerable, since no adverse effects were reported throughout the 6-week duration of the study. This toothpaste was positively appreciated by the patients, although not significantly different when compared with the control toothpaste in terms of PROMs. Regarding its efficacy, significantly higher reductions in plaque levels and BOP occurred at 3 and 6 weeks of use, when compared to the control toothpaste. These benefits were not related to an increase in tooth staining.

The safety of this CPC and cymenol-based toothpaste was assessed by both the occurrence of adverse and secondary effects. The patients using the test toothpaste did not experience any adverse effect at any time during the study, and the presence of staining was not statistically different to the control group. Although CPC has shown potential for inducing dental staining, this effect has been rarely reported in clinical studies [11], which may indicate a lower substantivity compared with CHX. CPC, when formulated as an oral hygiene product at concentrations ranging from 0.045% to 0.10%, has been shown to be safe and well tolerated [26]. Cymenol, as a tasteless isomer of thymol, has also been recognized as safe by the FDA [27]. In a study evaluating a toothpaste containing cymenol and zinc chloride, 14 out of 205 subjects reported mild adverse effects, although these did not cause any withdrawal from the study [13]. In the present study, patients' perceptions (PROMs) of the tested toothpastes, such as taste, aroma consistency or any disturbance, were assessed through a questionnaire. The results did not reveal any statistically significant difference between the groups. However, patients in the test group reported a sensation of lesser plaque removal efficacy, which did not correlate with the obtained clinical results, demonstrating significantly higher plaque reductions in the test group. It should also be noted that 40% of the subjects did not give an overall rating of the product from 1 to 10. This problem may be due to the fact the questionnaire was administered in a paper format of the questionnaire, and the subjects may have confused the question on product evaluation with the overall rating from 1 to 4. Administering the questionnaire in an online format would have made it possible to force subjects to answer all the questions.

In the present study, the use of the test toothpaste with CPC and cymenol resulted in higher reductions in PII levels when compared with the control group. This finding could be explained by the different active ingredients in the toothpastes. CPC possesses antimicrobial properties, particularly against a wide range of Gram-positive bacteria, fungi

and yeasts [28]. This antibacterial activity can be attributed to its cationic nature [29], which may prevent bacterial adhesion and hence, biofilm formation. On the other hand, cymenol exhibits direct antimicrobial effects against oral anaerobic species and can also reduce the production of metabolites, including glycolytic and proteolytic enzymes and thereby can reduce deleterious effects of certain microorganisms [30]. Moreover, when formulated as a mouth rinse, CPC and cymenol may act synergistically, by extending the substantivity of their action [14]. However, it is not easy to establish direct comparisons between the present study and others, due to the limited evidence available assessing CPC- and essential oil-containing toothpastes. A study evaluating the use of a mouth rinse with 0.05% CPC for 3 months in orthodontic patients found statistically significant differences in plaque levels between the test and control groups only in the first month [31]. In contrast, other studies evaluating mouth rinses containing 0.07% CPC [28,32] have demonstrated plaque reductions after 3 and 6 months, when compared to the placebo. When two mouth rinses containing CPC at concentrations of 0.075% and 0.10% were compared, both provided statically significant plaque reductions over six months of use [33]. In addition, numerous systematic reviews have investigated the effectiveness of mouth rinses containing CPC and essential oils [6,7,34], only essential oils [35] or CPC [11], demonstrating a statistically significant impact on plaque levels.

With regards to gingival inflammation, in the present study, both the test and control groups demonstrated reductions in BOMP, with minimal intergroup differences. Similar results in BOMP were observed with mouth rinses containing 0.05% [36] and 0.07% CPC [28], although these studies observed statistical differences in BOMP at 6 months, when compared with a placebo. Regarding the BOP changes in our study, both the test and control groups demonstrated statistically significant reductions and, more importantly, the changes between baseline and week 6 in the test group revealed a statistically significant higher reduction, when compared to the control group (test 0.19, SD = 0.13 and control 0.12, SD = 0.10, with a mean difference of 0.075, $p = 0.031$). In addition, an RCT evaluating 0.075% and 0.10% CPC mouth rinses observed a significant reduction in the gingival and bleeding indices, at 3 and 6 months, with a magnitude similar to CHX [33]. When formulated independently, both CPC and essential oils have demonstrated a significant impact on gingival indices, in 6-month RCTs [6]. Since clinical results could be influenced by the whole formulation of a given product [34], the results of the present study support that the CPC and cymenol combination, formulated in a toothpaste, demonstrate significant reductions in plaque and inflammation levels.

The limitations of the present study should also be disclosed: the convenience sample size of 30 patients per group is small, since it was selected following the primary aim, which was to assess safety and tolerability, and, also, the short study duration, that was also selected based on the primary aim of the study.

5. Conclusions

Within the limitations of the present study, it can be concluded that the use of a toothpaste containing cetylpyridinium chloride and cymenol was safe and well tolerated by the patients. Patients expressed their intention to purchase and recommend the product. The tested formulation yielded lower values for plaque and BOP, and demonstrated some additional benefits in terms of BOMP, without any associated staining. However, the relevance of the results obtained with this innovative combination should be confirmed with additional longer-term studies and with a larger number of subjects.

Author Contributions: Conceptualization, S.R., J.S., M.F., J.B., C.T., E.J. and D.H.; formal analysis, M.I.; investigation, V.V., F.L., P.M., M.I. and B.A.; resources, M.F., J.B., C.T. and E.J.; data curation, V.V., F.L., M.I. and D.H.; writing—original draft preparation, V.V., F.L., P.M., M.I., B.A. and D.H.; writing—review and editing, S.R., J.S., M.F., J.B., C.T. and E.J.; supervision, M.S. and D.H.; project administration, M.S. and D.H.; funding acquisition, D.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ISDIN S.A. (Barcelona, Spain), by means of a research contract (252-2021) with the Complutense University of Madrid (Spain).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki (Edinburgh revision of October 2000), and the protocol (version 1.1, 19 April 2021) was approved by the Ethics Committee of CEIC Hospital Clínico San Carlos, Madrid, Spain with the following registration number 21/262-EC_X on 21 April 2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data available on request due to restrictions. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy, data protection and ethical issues.

Acknowledgments: The authors declare that this study received funding from the company ISDIN, by means of a research contract with Complutense University of Madrid. Authors M.F., J.B., C.T. and E.J. are employed by the company ISDIN. Those authors had the following involvement with the study: conceptualization, resources and approval of the final version of the manuscript. The remaining authors (V.V., F.L., P.M., M.I., B.A., S.R., J.S., M.S. and D.H.) declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflicts of Interest: V.V., F.L., P.M., M.I., B.A., S.R., J.S., M.S. and D.H. declare no potential direct conflicts of interest related to the present work. M.F., J.B., C.T. and E.J. are employed at ISDIN S.A. (Barcelona, Spain), the company funding the study and the manufacturer of the tested product.

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