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Effect of a toothpaste containing triclosan, cetylpyridinium chloride, and essential oils on gingival status in schoolchildren: A randomized clinical pilot study

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Objective: This randomized double-blind in vivo pilot study has evaluated the effects of a toothpaste containing fluoride (control) versus toothpaste containing fluoride, triclosan, cetylpyridinium chloride and essential oils (experimental) in controlling supragingival dental plaque and bleeding on probing in a sample of healthy schoolchildren. **Method and Materials:** In total, 48 children (8 to 10 years) were selected and randomly divided into two groups (experimental and control), using the two different toothpastes twice a day for 2 minutes each for a 4-week period. The investigation included an evaluation of plaque quantity, using the Turesky modified Quigley-Hein method, and bleeding on probing that was recorded dichotomously. The unit of analysis was set at the gingival site level. Plaque Index and bleeding on probing were analyzed using distribution tables and chi-square test. A generalized estimating equation was used to estimate the parameters of a generalized linear model with a possible unknown correlation between outcomes. **Results:** In total, 40 schoolchildren completed the trial. Considering each group separately, a statistically significant difference in plaque scores was recorded for both treatments (z-test = 9.23, $P < .01$ for the experimental toothpaste; and z-test = 7.47, $P < .01$ for the control toothpaste). Nevertheless, the effect over time was higher for the experimental toothpaste than for the control one (3.38 vs 1.96). No statistically significant results were observed regarding bleeding on probing. **Conclusion:** The 4-week use of the experimental toothpaste seems to produce higher plaque reduction compared to fluoridated toothpaste without other antibacterial ingredients. This finding has to be confirmed in a larger study. *(doi: 10.3290/j.qi.a33530)*

Key words: cetylpyridinium chloride, dental plaque, essential oils, gingival bleeding, toothpaste, triclosan

In recent decades in Western countries, the widespread use of fluoridated toothpaste has played an important role in dental caries prevention and reduction.[1] The addition of other active ingredients aside from fluoride into toothpaste has become a common approach for periodontal disease control.[2,3] Dental plaque is the primary cause of periodontal disease, which, in its initial phase, occurs as gingivitis; mechanical plaque removal

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is able to prevent gingivitis, maintaining periodontal health.[4]

Epidemiologic data on the gingival status of Italian schoolchildren are quite scarce; at the age of 12 years, 52.5% of the children have unhealthy gingival conditions (Community Periodontal Index ≠ 0); 23.8% have bleeding gums and 28.7% have calculus.[5] High plaque and gingival scores were also recorded in schoolchildren with low socioeconomic background.[6] This situation calls for preventive strategies, as it has also been reported that even after education and motivation programs of oral hygiene methods, children reduce with time their compliance with proper toothbrushing.[7] Although chlorhexidine appears to be the most effective agent for dental plaque control, a significant reduction of its antiplaque potential may be observed when it is formulated into toothpaste;[8] in addition, the use of chlorhexidine-containing products for oral hygiene is not recommended for young children due to the risk of ingestion.[9] Moreover, the addition of different effective and safe antibacterial ingredients to toothpastes can represent an easy and low-cost strategy to improve gingival status.[10,11] Antimicrobial toothpastes have been marketed to provide additional anti-plaque/anti-gingivitis activity when used daily as an adjunct to a mechanical oral hygiene regimen.[12,13]

Triclosan is a non-ionic bisphenolic, wide-spectrum antibacterial agent with low toxicity that can be included in toothpastes; nevertheless, no evidence of its efficacy alone in the control of caries or plaque/gingivitis is available.[14,15] Triclosan affects bacterial growth and inhibits the production of prostaglandin E2 (PGE2) and leukotrienes in human gingival fibroblasts.[16]

Another broad-spectrum antimicrobial agent, cetylpyridinium chloride (CPC), an amphiphilic quaternary compound, is contained in mouthrinses and toothpastes in order to control supragingival plaque and gingivitis.[17,18] The CPC is retained in the oral environment through surfactant chains and cationic charges that are adsorbed to intraoral surfaces, binding bacterial cells and reducing microbial adhesion to oral surfaces.[19,20]

Essential oils (EO) from many different plants have been proposed and tested as promising agents in the treatment of oral diseases, showing a broad antimicrobial action against many different bacteria from the oral environment.[21] EO are used in mouthwashes, especially as an adjunct to brushing, due to their effectiveness in controlling plaque and gingivitis.[22] The antibacterial activity of EO is related to the destruction of the bacterial walls and the inhibition of the enzymatic activity.[3] EO mouthrinse has been shown to increase plaque and gingivitis reductions compared to CPC mouthrinse.[23]

The present study is part of a larger interventional study on the efficacy of triclosan, CPC, and EO as antiplaque agents. In order to decide if this study might be worthwhile, a randomized double-blind in vivo pilot study was designed to validate the hypothesis that a toothpaste containing fluoride, triclosan, CPC, and EO is more effective in controlling supragingival dental plaque and bleeding on probing (BOP) than a toothpaste containing fluoride in a sample of healthy schoolchildren.

METHOD AND MATERIALS

Study design and sampling

The study design followed the Declaration of Helsinki, and was approved by the Ethics Committee of the University of Sassari (registration no. 237/2014). Power analysis, using a one-sided confidence interval (CI), was performed to identify a sample size that gives reasonable confidence that this pilot trial is sufficiently large to enable a decision to be made about proceeding to a larger trial. The standardized effect was set at 0.30 with a sample size of 17 subjects and an upper 83% one-sided confidence limit of 0.41.[24]

The study was conducted from March to May 2014. All children (N = 92) aged 8 to 10 years from a primary school located in Sassari (Italy) suburban area were invited to participate. An information leaflet, explaining the aim of the study and requesting their child's participation with signed consent, was given to parents or guardians. Only children with parents' signed consent were called for examination (51 children).

Inclusion criteria were:

- good general health
- between 8 and 10 years of age

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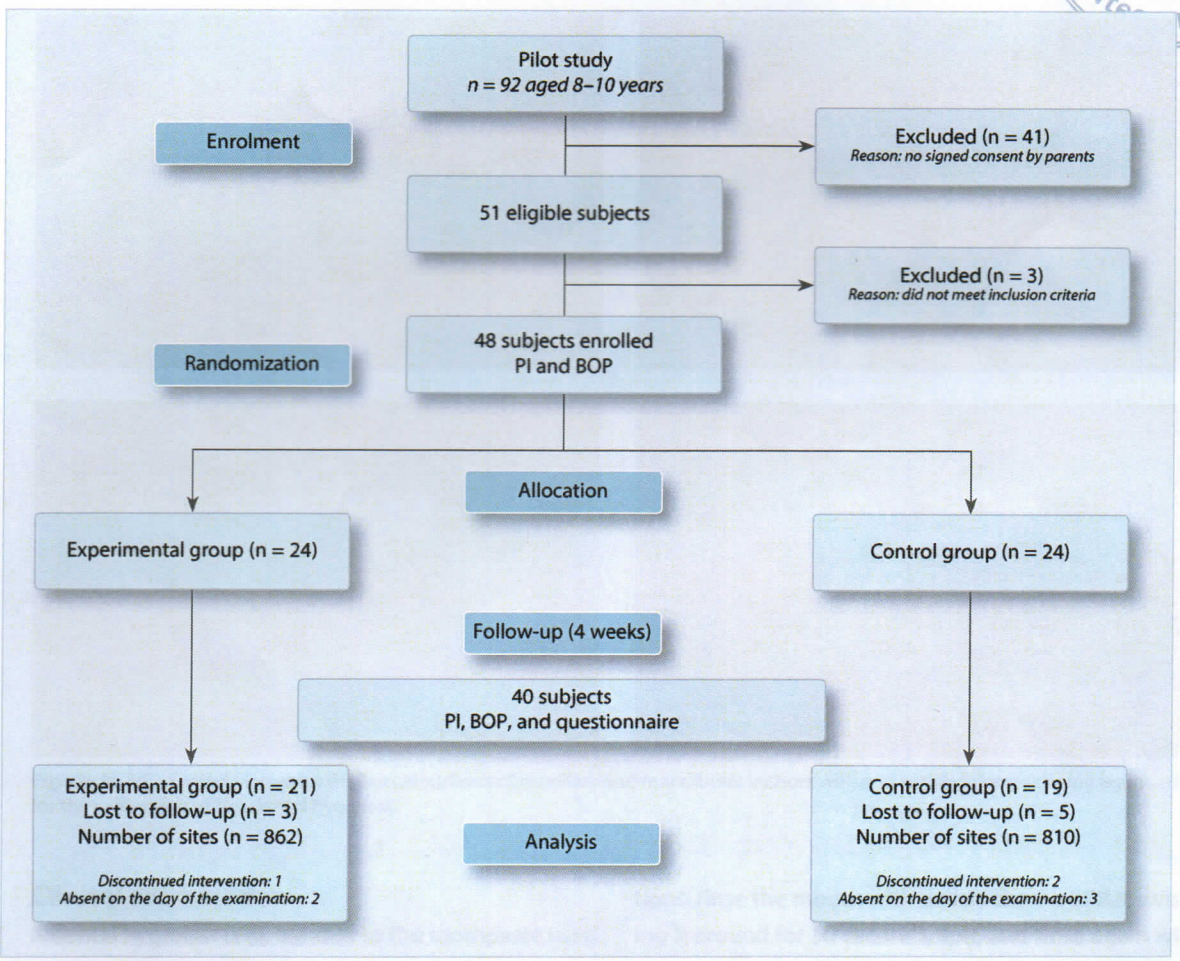


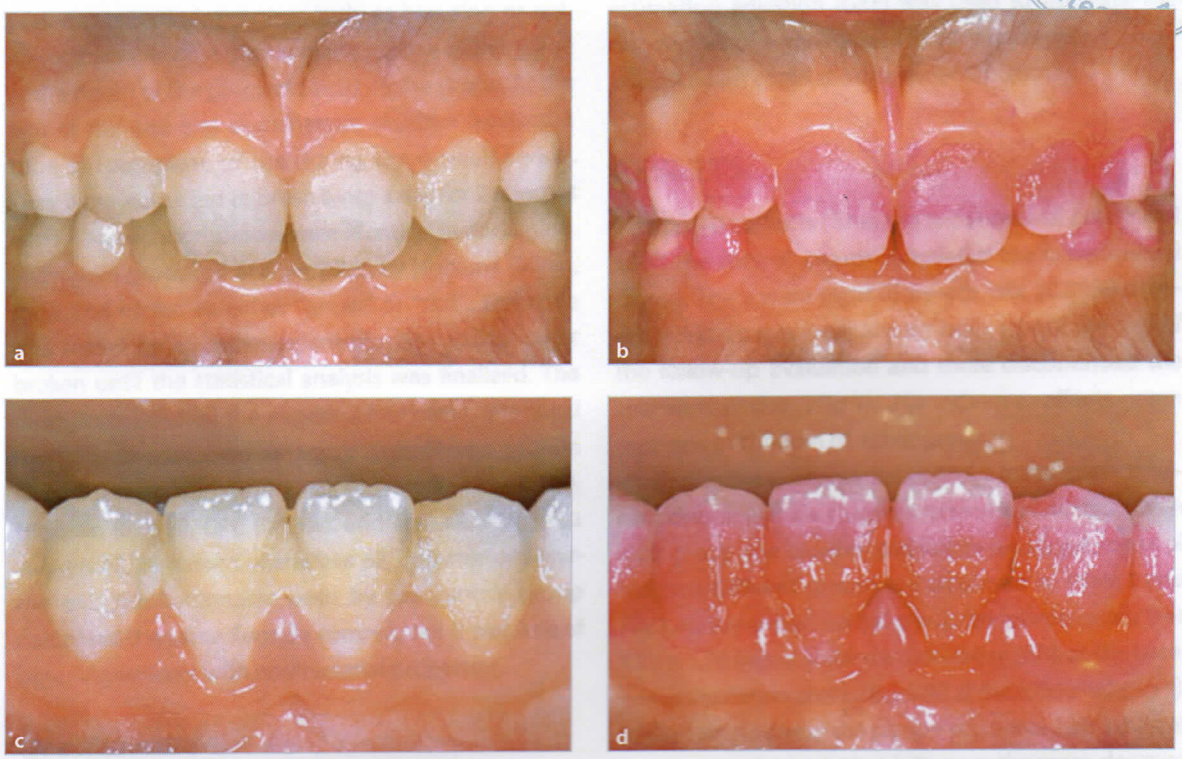
Fig 1 Flow chart of the study, following the CONSORT guidelines.

- the absence of a fixed orthodontic appliance
- the absence of carious and/or not-carious enamel defects in the buccal and lingual surfaces of the teeth that may facilitate the retention of dental plaque.

In total, 48 children were selected. The participants were compiled into a list and a list of true random numbers was generated (www.random.org). The generator was set to produce a true individual random sequence of "1" and "2", where "1" represented the group that would use a fluoridated toothpaste without other antibacterial ingredients (control group) and "2" represented the group that would use a fluoridated toothpaste containing triclosan, CPC, and EO (experimental group).

The investigation had a randomized, placebo-controlled study design (Fig 1), including an evaluation of plaque quantity and BOP, with an experimental period of 4 weeks. The two outcomes were evaluated at baseline (t_0) and after 4 weeks (t_1) regarding the use of the two toothpastes. All examinations were performed at school under the supervision of a teacher. Children were asked to abstain from oral hygiene procedures in the previous 24 hours and from eating and drinking in the previous 2 hours before the oral examination.

The use of other products for oral hygiene (ie, mouthrinse and/or toothpastes different from those administered) was forbidden during the entire experimental period.



Figs 2a to 2d Dental plaque on the buccal surfaces of maxillary and mandibular incisors with and without the disclosing agent, used for the calibration of the dental hygienist.

Clinical examination

A dental hygienist (VB), blinded to the toothpaste used by the children, performed the oral examination; she was calibrated 2 weeks before the start of the study. For calibration, five patients, not included in the study sample, were examined (plaque score recording and BOP), then photographs were taken of all buccal, lingual, and palatal surfaces using a Dental Eye II (Yashica) camera with standard photographic technique and exposure, a mouth mirror, and lip retractors (Fig 2).[25] One week before the clinical examination, the dental hygienist evaluated the scores of the dental surfaces in the selected photographs, according to the index used. A reliability test was performed to assess the intra-examiner performance, with a high level of concordance (Cohen-Kappa = 0.86).

Before the oral examination, all subjects used a dental disclosing tablet (Red-Cote, D&C Red #28, 1.5% w/w, Sunstar Suisse), following the manufacturer's directions: rinse the mouth with water, chew a tablet swishing it around for 30 seconds, spit, and rinse again with water. Plaque was scored using Quigley-Hein modified by Turesky (TQHPI),[26] using a plane mirror (No. 5, diameter 24 mm; Hu-Friedy) and a 1-mm periodontal probe (CP-15,5B Qulix; Hu-Friedy) under optimal artificial lighting. With the TQHPI, buccal and lingual surfaces were scored. Scoring was performed as follows:

- 0, no plaque/debris
- 1, separate flecks of plaque at the cervical margin of the tooth
- 2, a thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth
- 3, a band of plaque wider than 1 mm but covering less than one third of the crown of the tooth
- 4, plaque covering at least one third but less than two thirds of the crown of the tooth
- 5, plaque covering two thirds or more of the crown of the tooth.

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BOP was recorded dichotomously as bleeding or not, 30 seconds after the gentle manipulation of the tissue at the depth of the gingival sulcus by the probe. At the end of the first oral examination, children received a tube of toothpaste to use during the 4-week period. They were instructed to brush twice a day for 2 minutes each. The two toothpastes were identical in packaging. They were produced and supplied by Hobama (Milan, Italy) and coded as either "green" or "red". The code was sealed by an independent monitor and was not broken until the statistical analysis was finalized. The toothpaste used by the experimental group (Hobagel toothpaste) contained as active ingredients: sodium monofluorophosphate (0.76%), sodium fluoride (0.1%), triclosan (0.05%), CPC (0.03%), and some EO (*Thymus vulgaris* oil, *Malaleuca alternifolia* oil, *Commiphora myrrha* oil). The toothpaste used by the control group contained the same fluoride content, but it was free of other ingredients with antibacterial properties.

Questionnaire

A questionnaire was submitted to children at follow-up examination with five questions related to usage of the toothpastes and negative side effects such as taste disturbance or allergy development.

Statistical methods and data analysis

All the data were entered into an Excel (Microsoft) spreadsheet prepared ad hoc. Statistical analysis was performed using STATA SE 10.1 software (StataCorp). The unit of analysis was set at the gingival level, with a mean count of gingival sites in the experimental group equal to 44.20 sites and 45.33 sites in the control group; this small difference was due to the slight difference in the number of teeth present in the two groups ($P = .43$).

The Plaque Index (PI) and BOP were calculated in the two groups. The comparison between baseline and follow-up was carried out with the z-test; the comparison between baseline and follow-up at each plaque score was carried with a chi-square analysis for proportion or with the Fisher exact test. The mean and standard deviation of the PI for each site was also calculated and a paired *t* test was run. Moreover, a generalized estimating equation (GEE) was used to estimate the parameters of a generalized linear model with a possible unknown correlation between outcomes.

RESULTS

In total, 40 subjects (21 in the experimental group and 19 in the control group) completed the experimental period. There were eight (drop-out rate 20.00%) drop-out subjects: five were absent from school the day of the follow-up evaluation and three discontinued the toothpaste use (Fig 1). No adverse effects were reported in children of either group.

During the experimental period, a statistically significant decrease in the plaque scores was observed within each group. The baseline and follow-up plaque scores of the two different groups are displayed in Table 1 and Fig 3. In the experimental group the plaque score was 1.72 ± 1.70 (mean ± standard deviation [SD]) at the baseline examination and 1.18 ± 1.92 at the follow-up (Fig 4). In the control group the plaque score was 1.38 ± 1.70 at baseline and 1.10 ± 1.85 at follow-up; the mean decrease was 0.54 ± 1.26 in the experimental group and 0.28 ± 1.04 in the control one ($t = -0.91$; $P = .19$).

A statistically significant difference in TQHPI scores recorded at baseline between the two groups ($P = .04$) was observed. Considering each group separately, a statistically significant reduction in plaque scores was recorded for both treatments during the experimental period (z-test = 9.23, $P < .01$ in the group using the experimental toothpaste; and z-test = 7.47, $P < .01$ in the group using the control toothpaste). In the experimental group the comparison between baseline and follow-up scores revealed a statistically significant difference ($P < .01$ for scores 0, 2, 3, and 4 and $P < .05$ for score 1). A similar figure was recorded in the group using the control toothpaste, but only for scores 0, 2, and 3 ($P < .01$ for scores 2 and 3, and $P < .05$ for score 0). In the experimental group, a statistically significant increase of TQHPI scores 0 and 1 ($P < .01$) and a reduction of scores 2, 3, and 4 ($P < .01$) was observed compared to the control group (results not shown).

Table 2 displays the GEE for PI. The use of both toothpastes produced a statistically significant effect

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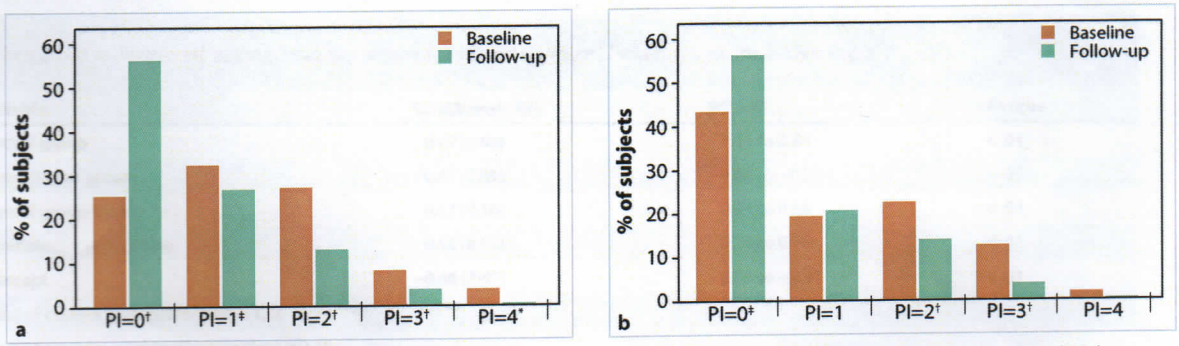
Table 1 Distribution of Quigley-Hein modified by Turesky plaque scores in the two groups; the unit of analysis was the number of sites (n = 862 in the tested group and n = 810 in control group)

	Baseline (mean ± SD)	**Follow-up (mean ± SD)**	**z test**	***P***			
	1.72 ± 1.70	1.18 ± 1.92	9.23	.01			
				Follow-up			
	Plaque Index (PI)	**Baseline**	**PI = 0 (n)**	**PI = 1 (n)**	**PI = 2 (n)**	**PI = 3 (n)**	**PI = 4 (n)**
Experimental group	**PI = 0**	219† (25.40)	148	62	7	1	1
	PI = 1	278‡ (32.25)	204	56	15	3	0
	PI = 2	233† (27.03)	89	81	51	11	1
	PI = 3	100† (8.11)	34	25	26	13	2
	PI = 4	32* (3.71)	9	6	16	4	0
	Total follow-up [n (%)]	862 (100.00)	484† (56.16)	230‡ (26.68)	112† (12.90)	32† (3.72)	4* (0.46)
	Baseline (mean ± SD)	**Follow-up (mean ± SD)**	**z test**	***P***			
	1.38 ± 1.70	1.10 ± 1.85	7.47	.01			
				Follow-up			
	Plaque Index (PI)	**Baseline**	**PI = 0 (n)**	**PI = 1 (n)**	**PI = 2 (n)**	**PI = 3 (n)**	**PI = 4 (n)**
Control group	**PI = 0**	351‡ (43.33)	221	79	49	2	0
	PI = 1	158 (19.51)	116	30	12	0	0
	PI = 2	183† (22.59)	86	35	50	12	0
	PI = 3	102† (12.59)	32	20	35	12	3
	PI = 4	16 (1.98)	1	4	6	5	0
	Total follow-up [n (%)]	810 (100.00)	456‡ (56.31)	168 (20.74)	112† (13.84)	31† (3.84)	3 (0.37)

*Fisher exact test, $P < .01$.
†Chi square for proportion $P < .01$.
‡Chi-square for proportion $P < .05$.



Figs 3a and 3b Baseline and follow-up plaque scores (PI 0, 1, 2, 3, and 4) in the experimental *(a)* and control *(b)* groups (*Fisher exact test, $P < .01$; †chi-square test for proportion, $P < .01$; ‡chi-square test for proportion, $P < .05$).

(coefficient 0.67, $P < .01$ for the control toothpaste; and coefficient −0.47, $P < .01$ for the experimental toothpaste); nevertheless, the effect over time was greater in the group using the experimental toothpaste (effect of control toothpaste/time 1.96 compared to 3.38 for the experimental one).

No statistically significant results were observed from the analysis of BOP (Table 3). Very few sites were bleeding at the baseline examination in both groups and no differences were observed into the two groups at baseline/follow-up comparison ($P = .08$ in the experimental group and $P = .14$ in the control group).

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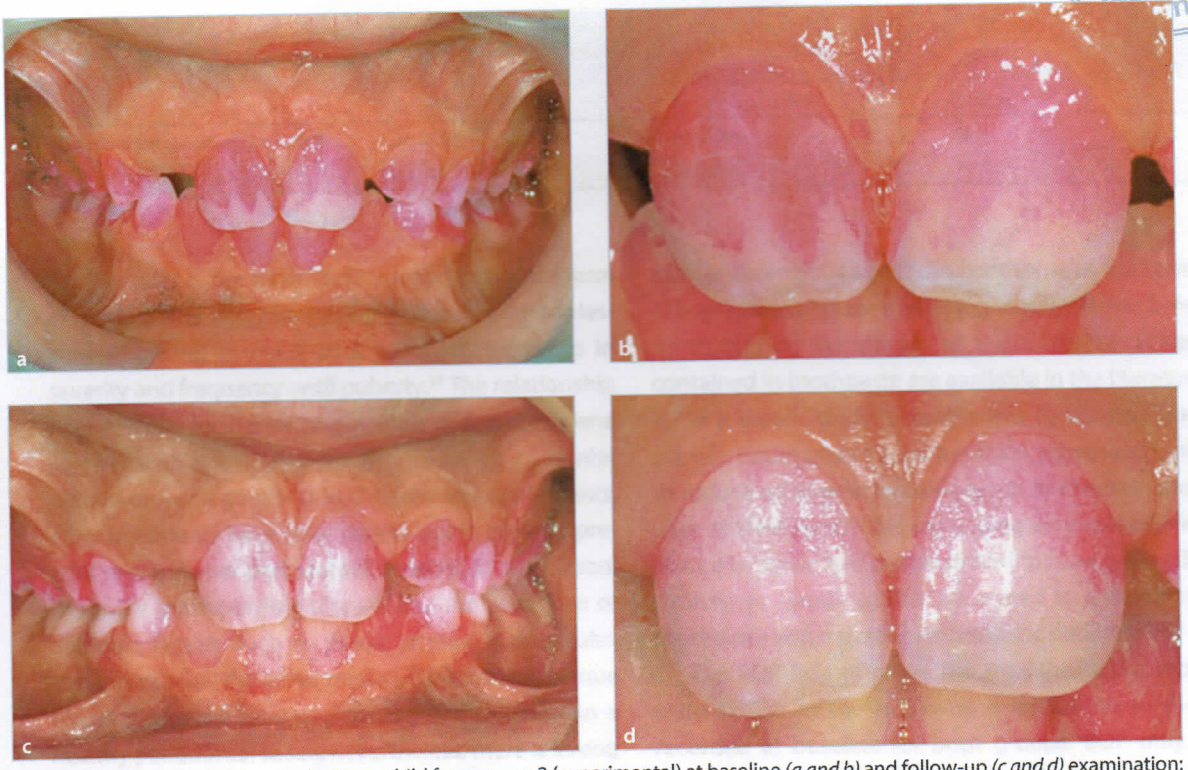

Figs 4a to 4d Dental plaque in the same child from group 2 (experimental) at baseline *(a and b)* and follow-up *(c and d)* examination: view of the whole mouth and maxillary central incisors.

Table 2 Generalized estimating equation for Plaque Index showing a cumulative logit

Variable	Coefficient (SE)	95% CI	*P* value
Control group	0.67 (0.08)	0.51 to 0.83	< .01
Experimental group	−0.47 (0.09)	−0.65 to −0.29	< .01
Control group/time	0.67 (0.10)	0.54 to 0.84	< .01
Experimental group/time	0.54 (0.11)	0.33 to 0.76	< .01
Intercept	−0.44 (0-07)	−0.58 to −0.31	< .01
Effect of control toothpaste/time = 1.96			
Effect of experimental toothpaste/time = 3.38			

DISCUSSION

The present pilot study was intended to evaluate the efficacy of a toothpaste containing fluoride, CPC, and EO in reducing supragingival dental plaque and BOP compared to a fluoride toothpaste without any other antibacterial ingredients in a sample of healthy schoolchildren. The main significant result concerned the quantity of plaque; both toothpastes produced a statistically significant reduction in plaque scores after 4 weeks of use, but the toothpaste containing the antibacterial agents seems to produce a significantly greater decrease in plaque quantity than the control toothpaste, and this finding will be verified in a future larger study.

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Table 3 Bleeding on probing distribution of affected sites in the two groups

	Baseline [n (%)]	Follow-up [n (%)]	*P* value
Experimental group (sites n = 862)	70 (8.12)	61 (7.08)	.08
Control group (sites n = 810)	33 (4.07)	35 (4.32)	.14

Gingivitis is the most common and prevalent form of periodontal disease among children and adolescents. Starting in childhood, gingivitis increases in severity and frequency until puberty.[27] The relationship between the incidence of gingivitis and oral hygiene level is well known.[28] With the presence of dental plaque-induced gingivitis in children and adolescents, early treatments have to be carried out in order to prevent more severe periodontal diseases in adulthood. Gingivitis is a reversible disease and the reduction of etiologic factors such as dental plaque and calculus reduces or heals the inflammation.[29] Mechanical plaque removal through brushing contributes to maintain a healthy periodontal tissue; nevertheless, there is strong evidence that the use of antibacterial agents in addition to toothbrushing contributes to plaque control and to prevent gingivitis.[2,3] An increased number of products such as mouthrinses, gels, and toothpastes containing chemotherapeutic agents have been proposed in the marketplace. To the best of the authors' knowledge, studies investigating the anti-plaque effect of a toothpaste containing triclosan, CPC, and EO in the same product has not yet been published.

The effect of toothpastes containing triclosan on plaque and gingival inflammation is the topic of a large number of studies.[14,15,30] A Cochrane meta-analysis reveals that there exists moderate-quality evidence that, in addition to fluoride, the antibacterial agent reduces plaque, gingival inflammation, and gingival bleeding when compared with fluoride toothpastes without triclosan/copolymer.[14] In addition, the association of two triclosan products (toothpaste and mouthrinse) showed a statistically significant higher plaque reduction compared to the use of a single product.[31]

The efficacy of mouthrinse containing CPC in plaque control is well documented: the broad spectrum antimicrobial agent can contribute to the control of supragingival plaque and gingivitis.[32,33] Nevertheless, no studies on the efficacy of the antibacterial agent contained in toothpaste are available in the literature.

In a recent study, a toothpaste containing *Lippia sidoides Cham* essential oil has been shown to be able to reduce cariogenic bacteria levels in children after 5 days of treatment.[34] Gel containing essential oil *Melaleuca alternifolia* was demonstrated to have good effectiveness in plaque control compared to a fluoridated toothpaste when used in orthodontic patients.[22]

The BOP fails to prove the hypothesis: the scores were similar at the follow-up examination to those recorded at baseline for both groups. BOP may be interpreted as a diagnostic test for the existence of a subepithelial infiltrate and gingival inflammation.[35] BOP was not modified by the treatment probably due to the low number of participants and/or the short duration of the experiment. The low prevalence of BOP recorded in the sample is similar to that found in other populations of schoolchildren.[36] Nevertheless, the two groups showed at baseline evaluation a nonhomogenous distribution regarding this parameter. This finding is probably related to two factors: the small number of subjects since this was a pilot study, and the sample selection criteria, as the two clinical parameters considered (supragingival plaque amount and BOP) were not evaluated for the enrolment. This is a possible weakness in the study. Nevertheless, since it was a pilot study, the aim was to verify if the studied product, toothpaste containing four different classes of antibacterial agents, had an effect in supragingival plaque reduction greater than that of fluoridated toothpaste in a sample of children. Generalization of the results of the study are not possible, as this report was designed as a pilot study; since a positive effect on gingival health of

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the experimental toothpaste was speculated, a large study will be designed and performed in the future.

CONCLUSION

The results of this double-blind clinical pilot study suggest that a toothpaste containing fluoride, triclosan, CPC, and EO seem to produce higher plaque reduction compared to a fluoridated toothpaste without other antibacterial ingredients during an experimental period of four weeks. These findings need to be confirmed in a larger study.

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