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ORIGINAL ARTICLE

The plaque- and gingivitis-inhibiting capacity of a commercially available essential oil product. A parallel, split-mouth, single blind, randomized, placebo-controlled clinical study

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Abstract

Background. Studies have reported commercially available essential oils with convincing plaque and gingivitis preventing properties. However, no tests have compared these essential oils, i.e. Listerine®, against their true vehicle controls. ***Objective.*** To compare the plaque and gingivitis inhibiting effect of a commercially-available essential oil (Listerine® Total Care) to a negative (22% hydro-alcohol solution) and a positive (0.2% chlorhexidine (CHX)) control in an experimental gingivitis model. ***Materials and methods.*** In three groups of 15 healthy volunteers, experimental gingivitis was induced and monitored over 21 days, simultaneously treated with Listerine® Total Care (test), 22% hydro-alcohol solution (negative control) and 0.2% chlorhexidine solution (positive control), respectively. The upper right quadrant of each individual received mouthwash only, whereas the upper left quadrant was subject to both rinses and mechanical oral hygiene. Plaque, gingivitis and side-effects were assessed at day 7, 14 and 21. ***Results.*** After 21 days, the chlorhexidine group showed significantly lower average plaque and gingivitis scores than the Listerine® and alcohol groups, whereas there was little difference between the two latter. ***Conclusion.*** Listerine® Total Care had no statistically significant effect on plaque formation as compared to its vehicle control.

Key Words: *anti-plaque agent, dental plaque, gingivitis, Listerine®*

Introduction

Systematic tooth brushing and adequate interdental cleaning, as well as regular follow-up by dentists or hygienists, is generally enough to prevent dental plaque-related diseases [1]. Gingivitis [2] is reversible if the supragingival dental plaque is removed [3], but left untreated, it may be the precursor [4] of and risk factor [5] for chronic destructive periodontal disease. Therefore, the pharmaceutical industry, as well as the dental profession, has searched for chemical ways to prevent dental plaque formation [6]. As a consequence, a number of rinsing agents for daily use have been developed, of which the essential oil product Listerine® is the most studied and celebrated.

Listerine® is a group of alcohol containing mouthwashes suggested to be potent inhibitors of plaque formation [7–18]. However, most of the scientific documentation of Listerine® has been obtained with 5% hydro-alcohol solutions as the negative control [9,10,12–17]. The commercially available Listerine® products contain from 21.6–26.9% alcohol [18], except a newly introduced alcohol-free version (Listerine® Zero). Therefore, to avoid research bias, testing the efficacy of Listerine® products should be done against its alcohol vehicle as the negative control.

The aim of the present study was to test the clinical effect of Listerine®, on plaque formation as primary, and gingivitis as secondary end-point, with or without mechanical oral hygiene, in a modified experimental gingivitis model, with 22% hydro-alcohol as the negative (placebo) and 0.2% chlorhexidine (CHX) solution as a positive control.

Materials and methods

Fifty-two dental hygienist, medical and dental students volunteered for the study (Figure 1). Prior to the start of the study, seven withdrew for personal reasons, resulting in 45 participants signing the

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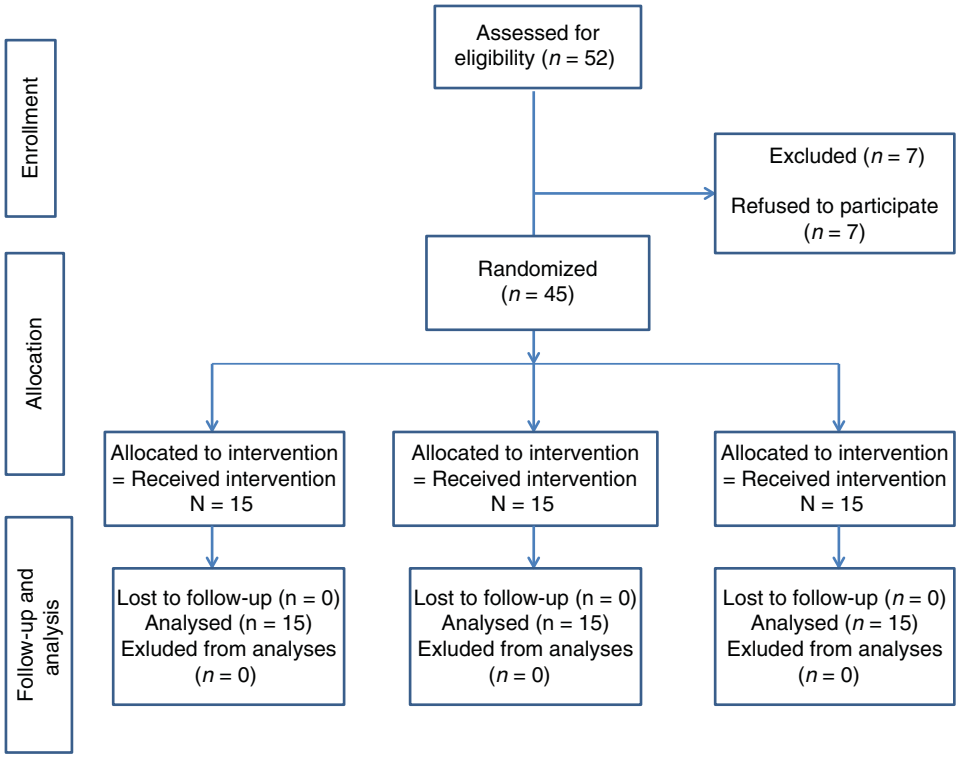

Figure 1. Flow diagram showing the steps in the identification of participants of the trial.

informed consent form. Mean age was 25.0 ± 3.2 years and 57.8% were females. They were given a minor economic compensation for the inconvenience. Following an interview, hematological diagnosis and a thorough clinical oral examination, consenting participants were included or excluded.

Inclusion criteria comprised healthy subjects from both genders, aged between 18–38 years, having at least three of the following teeth in maxillary right and left quadrant: The canine, 1st bicuspid, 2nd bicuspid, 1st molar, healthy gingiva and periodontium, being non-smokers and signing a written informed consent. Exclusion criteria comprised pregnancy, lactation, any chronic diseases, clinical signs or symptoms of acute infection in the oral cavity, any prescribed or non-prescription systemic or topical medication, except oral contraceptives, hematological and clinical parameters judged as unacceptable by the principal investigator (HRP), use of systemic antibiotics the last 3 months prior to the start of the study, history of alcohol or drug abuse or participation in other clinical studies in the last 4 weeks.

The study was approved by the Norwegian Social Science Data Services and Regional Committee for Medical Research Ethics, South East Norway in 2010 (REK.2010/1110-6). It followed the Consort 2010 guidelines (http://www.consort-statement.org/consort-statement/overview0/).

Upon informed request, the Norwegian product manager from the manufacturer suggested the best suited Listerine® product for this study to be Listerine® Total Care and gave explicit instructions for its correct use. The product was purchased from a local pharmacy. CHX was made from 20% CHX-gluconate diluted in water and used as described [19,20]. The alcohol content of the CHX solution was as in the corresponding commercial product and the final concentration of CHX-gluconate was as in the commercially-available product; 0.2%. The hydro-alcohol solution was made from 96% ethanol diluted with water to the final concentration of 22%.

Randomization was carried out using a computer-generated random allocation table [21] assigning the participants to three study groups containing 15 subjects each: Group 1 was instructed to rinse for 30 s twice daily with 20 ml of Listerine® Total Care as recommended by the manufacturer. Group 2 was instructed to rinse for 30 s twice daily with 20 ml of the 22% hydro-alcohol solution. Group 3 was instructed to rinse for 60 s with 10 ml 0.2% CHX twice daily. A separate periodontist, outside the project team, performed the randomization and distributed the rinsing solutions and instructions.

Setting the baseline dental plaque score to zero was done by giving all 45 participants a professional tooth cleaning with rubber cup, pumice paste and dental floss prior to the start of the study. Individual plastic tooth guards were produced to fit the teeth in the upper right quadrant (Q1) [22]. Together with this individual tooth guard, the students were given identical prophylaxis packs containing a soft texture tooth brush, inter-dental floss and dentifrice. In addition

Table I. Plaque index after 1, 2 and 3 weeks–rinsing only quadrant (Q1).

	1 week			2 weeks			3 weeks		
	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All
Listerine® Total Care	0.54 ± 0.45	1.28 ± 0.49	0.91 ± 0.46	0.53 ± 0.44	1.25 ± 0.50	0.89 ± 0.46	0.50 ± 0.49	1.13 ± 0.66	0.81 ± 0.56
Alcohol	0.59 ± 0.32	1.48 ± 0.37	1.03 ± 0.29	0.67 ± 0.36	1.53 ± 0.40	1.10 ± 0.37	0.73 ± 0.41	1.55 ± 0.44	1.14 ± 0.41
CHX	0.15 ± 0.25*	0.61 ± 0.51*	0.38 ± 0.36*	0.08 ± 0.19*	0.42 ± 0.40*	0.25 ± 0.29*	0.10 ± 0.18*	0.51 ± 0.27*	0.30 ± 0.21*

Mean ± SD.
*Significant $p < 0.05$.

they received ninety 0.5 mg fluoride tablets in order to prevent development of early carious lesions during the course of the study. They were instructed to replace their previous oral hygiene remedies with the received prophylaxis pack, in which the tooth guard was attached to the tooth brush so that the use of this always was remembered when using the brush.

The participants were instructed to insert the tooth guard in Q1 every time they brushed their teeth and to perform a mechanical oral hygiene routine twice daily. After brushing properly, they were instructed to rinse for 30 s with water before removing the tooth guard and then rinse again for 30 s with water without the mouth guard. Following this procedure the participants rinsed, as instructed, with the solution they randomly had been assigned to use. This oral hygiene routine was repeated for 21 days. The study design and stringency of logistics [22] ensured that the study participants served as their own controls, as one quadrant was exposed to chemical rinsing only (Q1) and the upper left quadrant (Q2) received traditional tooth brushing and inter-dental cleaning in addition to the chemical rinsing. Following the last scoring at day 21, the participants received another professional tooth cleaning session before ending the study.

The study period was 21 days (14 October–5 November 2010), not comprising any special academic, religious or ethnic feasts or events which could jeopardize the collective behavior of the study population. All information, administration and data collection was performed at the Department of Periodontology, Institute of Clinical Odontology, Faculty of Dentistry, University of Oslo, Norway.

A team of three people were trained in the procedure of informing participants, receiving the test persons for evaluation, questionnaire and clinically monitoring them at all visits [22]. The principal investigator (HRP) managed all contact with the participants outside the scoring room. In between appointments, HRP kept in touch with the test persons by text messaging and E-mail. The success of this service was evident by zero no-shows at the clinic.

Preceding every examination, HRP interviewed the participant about compliance and received verbal complaints and descriptions of side-effects. Before entering the scoring room, HRP ordered the participants to refrain from any conversation with the scoring scientists inside. The recorders had been instructed likewise.

In the scoring room, two researchers obtained the clinical data. At days 7, 14 and 21, Plaque Index (Pl.I) and Gingival Index (GI) [23] were recorded on the mesial, buccal, distal and palatal aspects of teeth 16, 15, 14, 13 and 23, 24, 25, 26. Adverse events, like discoloration observed during the clinical examination (yes/no) and clinically visible oral mucosal reactions were registered. All clinical registrations were performed by the same experienced periodontist, leaving her colleague to register recordings on specially designed charts. HRP and the scoring crew were kept blind to the group allocation of the participants at all times.

Statistical analyses

The total number of participants was 45, with 15 subjects in each group (using a two-sided *t*-test with 5% significance level); the test power to detect a true mean difference in GI and Pl.I was above 80%. This power analysis was based on the variable delta plaque (DP), meaning the difference in mean plaque between 1st and 2nd quadrant (test minus control quadrant). When comparing mean DP in two groups, a two-sided independent samples *t*-test was used, with 5% significance level. In the present study sample size was 15 patients in each group. Average standard deviation of DP in the three groups was 0.40. It may be shown that the test power to detect a mean difference of at least 0.40 in the present study when comparing two groups was 78%. As 80% test power is generally considered acceptable in clinical studies and the difference in mean DP between group 3 and group 1 was 0.49, the above calculation suggests that our study has acceptable test power.

Interval estimates of primary efficacy variables were constructed using 95% as the level of confidence and an overall significance level of 5% was used in the statistical tests. All tests were performed two-sided.

Table II. Gingival bleeding index after 1, 2 and 3 weeks–rinsing only quadrant (Q1).

	1 week			2 weeks			3 weeks		
	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All
Listerine® Total Care	0.72 ± 0.32	1.09 ± 0.42	1.02 ± 0.44	0.56 ± 0.32	1.34 ± 0.54	1.22 ± 0.48	0.76 ± 0.41	1.50 ± 0.46	1.29 ± 0.43
Alcohol	1.06 ± 0.38	1.27 ± 0.52	1.16 ± 0.37	0.90 ± 0.47	1.32 ± 0.41	1.11 ± 0.39	0.92 ± 0.40	1.77 ± 0.31	1.34 ± 0.27
CHX	0.94 ± 0.52	1.04 ± 0.42	0.88 ± 0.30	1.09 ± 0.50*	1.07 ± 0.43	0.81 ± 0.29*	1.07 ± 0.55	1.22 ± 0.32	0.99 ± 0.32*

Mean ± SD.
*Significant $p < 0.05$.

The statistical analyses were conducted using the software of SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL). The difference between groups at days 7, 14 and 21 were tested using independent samples *t*-test.

Results

Q1: Rinsing only quadrant

Rinsing with CHX resulted in an average plaque score of 0.30 after 21 days, which was significantly lower than the results in the two other groups (Table I). The difference in average plaque scores between Listerine® and alcohol control was not statistically significant at any time in the study. In the proximal sites, the CHX group had significantly lower plaque scores than Listerine® and alcohol groups after 21 days. The average plaque scores in the Listerine® and alcohol groups remained high throughout the study (Table I).

The GI scores were significantly lower in the CHX group after 21 days, compared to the alcohol and Listerine® groups (Table II), when mouthrinse was the only plaque-inhibiting procedure used. When comparing the gingival condition in the proximal sites only, there were no differences between the three mouthrinses.

Q2: Rinsing and mechanical oral hygiene quadrant

When tooth brushing, flossing *and* rinsing were performed in the same quadrant (Q2), the plaque scores were very low in all three groups (Table III). The GI scores in Q2 showed no statistically significant differences between the three mouthrinses (Table IV).

Adverse effects were registered in all three groups (data not shown). In the CHX group, discoloration, burning sensation and reduced taste were the most common complaints, whereas oral mucosal, tongue-tip and gingival burning sensation were the most common problems with Listerine® and alcohol. Except for the discoloration and reduced taste sensation of the CHX group, there was no statistical difference between groups regarding complaints of discomfort or clinical adverse effects during these 21 days.

Discussion

The findings of this study are in contrast to most clinical studies as well as an abundance of reviews on Listerine® [7,24–34]. In this study, CHX performed as the literature has portrayed it [35] and significantly better than Listerine® Total Care. The positive effect of CHX has been reported numerous times in the literature. The interesting comparison was between Listerine® and its 22% hydro-alcohol placebo solution, but in hindsight this study might have benefitted from one or two additional negative control group(s) rinsing with saline and/or water. This was discussed, but introducing another group(s) would have required another 15–30 test persons which would have been both too costly and difficult to recruit. On the other hand, most studies have compared Listerine® to 5% hydro-alcohol solution [9,10,12–17], saline or water [27,33,36,37], reporting significant differences. Therefore, the above

Table III. Plaque index after 1, 2 and 3 weeks–rinsing and brushing quadrant (Q2).

	1 week			2 weeks			3 weeks		
	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All
Listerine® Total Care	0.03 ± 0.09	0.26 ± 0.16	0.15 ± 0.10	0.03 ± 0.07	0.20 ± 0.26	0.12 ± 0.16	0.00 ± 0.00	0.10 ± 0.12	0.05 ± 0.06
Alcohol	0.07 ± 0.12	0.35 ± 0.16	0.21 ± 0.09	0.02 ± 0.07	0.28 ± 0.22	0.15 ± 0.13	0.05 ± 0.09	0.21 ± 0.19	0.13 ± 0.13
CHX	0.02 ± 0.04	0.16 ± 0.15	0.09 ± 0.08	0.00 ± 0.00	0.16 ± 0.09	0.03 ± 0.04	0.17 ± 0.07	0.06 ± 0.09	0.04 ± 0.05

Mean ± SD.
No statistically significant data.

Table IV. Gingival bleeding index after 1, 2 and 3 weeks–rinsing and brushing quadrant (Q2).

	1 week			2 weeks			3 weeks		
	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All	Bucc/Pal	Approx	All
Listerine® Total Care	0.48 ± 0.35	0.78 ± 0.40	0.63 ± 0.32	0.26 ± 0.33	0.73 ± 0.57	0.50 ± 0.43	0.28 ± 0.49	0.58 ± 0.63	0.43 ± 0.54
Alcohol	0.67 ± 0.42	0.89 ± 0.55	0.78 ± 0.37	0.41 ± 0.39	0.79 ± 0.53	0.60 ± 0.41	0.38 ± 0.54	0.73 ± 0.58	0.56 ± 0.50
CHX	0.39 ± 0.09	0.79 ± 0.47	0.59 ± 0.38	0.09 ± 0.19	0.47 ± 0.41	0.28 ± 0.26	0.03 ± 0.13	0.27 ± 0.29	0.15 ± 0.17

Mean ± SD.
No statistically significant data.

contemplated additional comparisons have repeatedly been made.

When reviewing the literature, very few studies indicate which type of Listerine® has been used as test and none of the studies applied a placebo with the same alcohol content as the test substance. Since the group of Listerine® products, from which the test solution of this study was selected, contains 21.6–26.9% alcohol [18], the correct placebo control is the vehicle for the essential oil (i.e. Listerine®). This is obviously a 21.6–26.9% hydro-alcohol solution. As mentioned above, studies on Listerine® have used water or saline controls [27,33,36,37] or more frequently 5% hydro-alcohol solution [9,10,12–17], whereas two used 10% hydro-alcohol solution [33,38]. Some reported 'vehicle control' or 'placebo' [39–41] without detailing the information further. Thus, the comparatively high alcohol content, rather than the essential oils as such, may have accounted for the slight antibacterial effect shown in these studies.

In the chemical/mechanical cleaning quadrant (Q2), the plaque index remained close to zero in all groups, suggesting that mechanical cleaning of teeth is sufficient to keep plaque and gingivitis scores at a minimum, which is in agreement with others [42]. Thus, sufficient oral hygiene for prevention of oral plaque-related diseases should be easily achieved with information, awareness and instruction and generally without the help of antibacterial rinses.

Among the self-reported side-effects, soreness of the gingiva and mucous membranes (burning sensation) were the most common complaints. Burning sensation was evenly distributed between the groups. Both Listerine® and the alcohol placebo contain high enough concentrations of alcohol (22%) to cause such side-effects with time and CHX is known to cause the same sensation even without its alcohol content [43]. In this respect, the repeated use of mouthwashes with high alcohol content has been questioned since it has been suggested to increase the risk of developing oral mucous membrane disorders like oral cancer [18,44,45].

The data collection team was blinded to the group allocation. The principal investigator (HRP) was not involved in data collection procedures, except verbally collecting compliance information and complaints on adverse effects. The complaints of discomfort and other side-effects were quite common and were not of a nature that could give away group allocation. However, some of the students might themselves have known, by recognizing the Listerine® taste, who rinsed with the test or control mouthrinses. In the interview preceding screening, all students were explicitly instructed not to talk among themselves about their possible group allocation. Thus, the study may be regarded as double blind, but, since the students might have communicated the mentioned differences, despite strict orders not to, the correct designation would be single-blind.

The population in the present study was dental hygienist, dental and medical students. They had a clear understanding of how to clean their teeth and probably more so than the general population. However, the use of the Q1 mouthguard eliminated this possible difference from the general population. As important was the compliance achieved with this dentate and hygiene-aware population. They were all non-smokers, thus staining or masked gingival inflammation due to smoking did not influence the results. Twenty-six (57%) of the subjects were females and of these 16 (62%) subjects had their menstruation during the study period. Variations in time frame of menstruation cycle is affected by contraceptives, which was used by 11 (42%) of these. The variation in hormonal status may, therefore, to some degree have influenced the gingivitis scores [46]. The compliance was excellent and, even if some of the participants complained about severe soreness of the tongue tip and oral mucosa during rinsing with the test and control solutions, they all reported to have performed according to protocol.

In the present study we used the modified [22] experimental gingivitis model over 3 weeks [3]. Several essential oil studies have lasted for 3–9 months [9–11]. There is no scientific evidence that a plaque-inhibiting mouthwash will perform better after several months than after 3 weeks. The experimental gingivitis model [3] has shown repeatedly that the plaque accumulation increases against a limit after 3 weeks and therefore this model, enhanced by the modification [22], should suffice in detecting

differences in plaque scores among the test and control groups.

This experiment was performed with Listerine® Total Care, as recommended by the manufacturer. However, it is shown in the EU-product information on Listerine® products that they contain the same ingredients with differences in color and taste, except for the alcohol-free version where propylenglycol has substituted alcohol as the solvent for the essential oils. So, basically, one product in the Listerine® series is comparable to the others in terms of claimed antibacterial contents.

In conclusion, testing the clinical efficacy of Listerine® Total Care on plaque formation and gingivitis in this modified experimental gingivitis model, with 22% hydro-alcohol and 0.2% CHX solutions as controls, no statistically significant antibacterial effect of Listerine® over its placebo vehicle was found. Neither Listerine® nor alcohol had any effect of clinical value to the user, since the amount of accumulated plaque after rinsing was still enough to cause any of the dental plaque-related diseases.

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