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Effect of Eucalyptus Extract Chewing Gum on Periodontal Health: A Double-Masked, Randomized Trial

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Background: Studies in vitro showed that eucalyptus extracts possess antibacterial activity against cariogenic and periodontopathic bacteria; however, the clinical effects with respect to periodontal health in humans remain unproven. The objective of this study was to evaluate the effect of chewing gum containing eucalyptus extract on periodontal health in a double-masked, randomized, controlled trial.

Methods: Healthy humans with gingivitis but not deep periodontal pockets were randomly assigned to the following groups: high-concentration group (n = 32): use of 0.6% eucalyptus extract chewing gum for 12 weeks (90 mg/day); low-concentration group (n = 32): use of 0.4% eucalyptus extract chewing gum for 12 weeks (60 mg/day); and placebo group (n = 33): use of chewing gum without eucalyptus extract for 12 weeks. Plaque accumulation (PLA), gingival index (GI), bleeding on probing (BOP), periodontal probing depth (PD), and clinical attachment level (CAL) were measured at weeks 0, 4, 8, 12, and 14. Significance was analyzed with repeated-measures two-way analysis of variance followed by the Games-Howell pairwise comparison test.

Results: The interaction between the effects of eucalyptus extract chewing gum and the intake period was statistically significant for PLA, GI, BOP, and PD but not for CAL. The low- and high-concentration groups exhibited statistically significant ($P < 0.05$) improvements compared to the placebo group for PLA, GI, BOP, and PD.

Conclusions: Eucalyptus extract chewing gum had a significant effect on PLA, GI, BOP, and PD. The use of eucalyptus extract chewing gum may promote periodontal health. *J Periodontol 2008;79:1378-1385.*

KEY WORDS

Chewing gum; eucalyptus; periodontal index; randomized controlled trial.

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Eucalyptus, which is native to Australia, is a widely planted genus. *Eucalyptus globulus* is a representative *Eucalyptus* species; its leaf is used for medicinal purposes and as a food source, e.g., tea, natural additives, and health foods. Ethanol extracts (60% ethanol) from *E. globulus* leaves reportedly possess antibacterial activity against various bacteria, including oral bacteria.[1-5] The extracts exhibit potent antibacterial activity against cariogenic bacteria, such as *Streptococcus mutans* and *Streptococcus sobrinus*; additionally, the extracts inhibit insoluble glucan synthesis by extracellular glucosyltransferase from *S. sobrinus*.[4] The anticaries activity of the extracts was also documented in the gnotobiotic BALB/cA mouse model.[6] Moreover, 60% ethanol extracts from the *E. globulus* leaf displayed antibacterial activity against several periodontopathic bacteria, including *Porphyromonas gingivalis* and *Prevotella intermedia*. In particular, among periodontopathic bacteria, the growth of *P. gingivalis* was strongly inhibited even with a low concentration (10 μg/ml) of eucalyptus extracts.[7]

Macrocarpals, which are polyphenols unique to eucalyptus, are major components of 60% ethanol extracts of *E. globulus* leaf; furthermore, these compounds exhibit several interesting biologic properties, such as antibacterial and antiviral

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activities,[8] antagonism of thromboxane A_2 and leukotriene D_4,[9] human immunodeficiency virus–reverse transcriptase inhibition,[10,11] and aldose reductase inhibition.[12] Macrocarpals A, B, C, D, H, I, and J and eucalypton were isolated in the mid-1990s.[3,4,13] Macrocarpals A, B, and C, which are major components, demonstrate relatively strong anticariogenic bacterial activity against *S. mutans* and *S. sobrinus* and inhibit glucosyltransferase produced by *S. sobrinus*.[4] Additionally, macrocarpals A, B, and C possess antiperiodontopathic bacterial activity against several periodontopathic microorganisms, e.g., *P. gingivalis*.[5] Macrocarpal C occurs in the greatest abundance in eucalyptus extract, and it exhibits the strongest antibacterial activity against periodontopathic bacteria.[5] Moreover, macrocarpals A, B, and C inhibit the activity of virulence factors of *P. gingivalis*, including Arg- and Lys-specific cysteine proteinases, as well as adhesion of the organism to saliva-coated hydroxyapatite beads.[5] Therefore, in ethanol extracts of eucalyptus leaf, macrocarpals A, B, and C (macrocarpal C in particular) are considered the primary antibacterial agents against cariogenic and periodontopathic bacteria.

Evidence derived from in vitro studies[4,7] indicates that 60% ethanol extracts of *E. globulus* leaf possess potent anticariogenic and antiperiodontopathic bacterial activities as described above; however, little is known about the clinical effects of eucalyptus extracts on periodontal health in humans. As a result, an in vivo examination of the effect of eucalyptus extract on periodontal health was warranted. In this study, a double-masked, randomized, controlled trial was conducted in humans using chewing gum containing eucalyptus extract. Subsequently, the effectiveness of eucalyptus on periodontal health was assessed.

MATERIALS AND METHODS

Subjects

Approval for this study was obtained from the Ethical Committee for Clinical Research, Osaka University Graduate School of Dentistry. Participants were recruited from February 2006 to June 2006. A total of 149 individuals, all of whom resided in the suburbs of Osaka, ranged in age from 20 to 49 years, and were concerned with their gingival status, applied for this study. Following receipt of a written informed consent form, participants were examined in terms of gingival index (GI)[14] and periodontal probing depth (PD) as described below; additionally, participants underwent a blood test and urinalysis at Osaka University Dental Hospital. Subjects were excluded from this investigation for the following reasons: antibiotic treatment or periodontal treatment within the previous 3 months, a history of systemic disease, abnormal findings of the blood test and/or urinalysis, <24 teeth, absence of gingivitis (GI = 0), or PD >6 mm, even at only one site. One hundred subjects (49 males and 51 females) with gingivitis were selected for this double-masked, randomized, controlled clinical study. Each individual was randomly assigned to one of the following groups by the study coordinator (Japan Medical Laboratory, Osaka, Japan): high-concentration group (n = 33) = use of 0.6% eucalyptus extract chewing gum for 12 weeks (90 mg/day); low-concentration group (n = 33) = use of 0.4% eucalyptus extract chewing gum for 12 weeks (60 mg/day); and placebo group (n = 34) = use of chewing gum without eucalyptus extract for 12 weeks. Subjects were divided into groups based on rank weighted by GI, age, and gender (10:8:7, respectively), after which they were allocated to three groups using a table of random numbers to equalize mean ± SD of GI, age, and gender. One hundred subjects received full-mouth supragingival scaling 2 weeks prior to the baseline examination; however, three subjects were excluded before the baseline examination because of personal reasons. Although one subject (in the high-concentration group), who left after the baseline examination, did not undergo examinations at weeks 4, 8, 12, and 14, his data at baseline were included for statistical analysis. As a result, the data of 97 subjects were analyzed: high-concentration group, n = 32, 16 males and 16 females, mean age, 33.72 years; low-concentration group, n = 32, 13 males and 19 females, mean age, 33.38 years; and placebo group, n = 33, 19 males and 14 females, mean age, 34.73. All participants were masked to the assignment, and no toothbrushing instruction was given for the duration of the study.

Chewing Gum

Eucalyptus extracts: *E. globulus* leaf, from which essential oil components were removed by steam distillation, was extracted with water at 80°C for 90 minutes. After filtration, 60% (weight/weight) ethanol was added to the residue, and the mixture was refluxed for 1 hour. The 60% ethanol extract was filtered and gum Arabic was added to the extract. The suspension was concentrated *in vacuo* and powdered with spray-dryer. The extracts contained 0.74%, 0.36%, and 0.8% of macrocarpals A, B, and C, respectively. Chewing gum used in this study was supplied by Lotte Central Laboratory. The components, other than the eucalyptus extracts, were identical to those of tablets of sugarless chewing gum currently on the market. The weight of each tablet was 1.5 g. The proportion of compounds contained in test gum (0.6% or 0.4% eucalyptus extract chewing gum) and placebo gum is presented in Table 1.

Study Design

A diagram depicting the flow of participants through the trial is shown in Figure 1. Two weeks prior to the use of chewing gum, all subjects received full-mouth

Table 1.

Proportion of Compounds in Gum

	Test Gum		
	High Concentration	Low Concentration	Placebo Gum
Eucalyptus extract (%)	0.6	0.4	0.0
Eucalyptus extract (mg/tablet)	9.0	6.0	0.0
Macrocarpal A (μg/tablet)	66.3	44.2	0.0
Macrocarpal B (μg/tablet)	32.4	21.6	0.0
Macrocarpal C (μg/tablet)	72.0	48.0	0.0
Carbohydrate (%)	80.5	80.7	81.1
Xylitol (mg/tablet)	666.0	666.0	666.0
Maltitol (mg/tablet)	508.0	511.0	511.0
Gum base (%)	13.7	13.7	13.7
Others (%)	5.2	5.2	5.2
Total (%)	100.0	100.0	100.0

Values are expressed as weight/weight (%).

supragingival scaling. During the intake period (12 weeks), subjects chewed two tablets of chewing gum for 5 minutes five times per day: the high-concentration group was exposed to 90 mg/day of eucalyptus extract, and the low-concentration group was exposed to 60 mg/day of eucalyptus extract. Subjects were instructed to chew the gum after the three main meals and between meals (two periods); thus, a designated time to chew the gum was not indicated. Periodontal and general examinations were conducted at baseline and at weeks 4, 8, 12, and 14.

Periodontal Examination

Clinical periodontal examinations were performed by five trained and calibrated examiners who were masked to the group assignments. The five examiners were calibrated using volunteers in the Faculty Hospital at Osaka University before and during the survey. In principle, the same examiner examined the same subjects throughout the trial; however, in some cases, the examiner examined subjects of whom the examiner was not in charge. The mean κ value among examiners was 0.7 for assessment of PD; calibrations for the assessment of other periodontal indices were not determined. The following clinical data were obtained from all subjects. Plaque accumulation (PLA) was measured according to the method described by Suzuki et al.[15] Briefly, upper right and lower left molars, upper left and lower right premolars, and upper left and lower right incisors were selected. Plaque was dyed with plaque-disclosing solution;‡ subsequently, the height of PLA from the gingival margin was measured at six sites (buccal, mesio-buccal, disto-buccal, lingual, mesio-lingual, and disto-lingual) in 0.5-mm increments using a University of North Carolina probe.§[16] The scores from the six areas of each tooth were added and divided by six, which yielded the PLA for the tooth. Summation of the scores for the teeth followed by division by six provided the mean PLA for each individual. GI, which was determined according to the method of Löe and Silness,[14] was recorded at four sites per tooth (buccal, lingual, mesial, and distal) using the same teeth as for PLA. The scores from the four areas of each tooth were added and divided by four to yield the GI for the tooth. Summation of the scores for the teeth followed by division by six provided the mean GI for the individual. Bleeding on probing (BOP) was recorded as positive when the buccal or lingual site bled within 20 seconds following removal of the probe tip in all teeth;[17] subsequently, the percentage of BOP-positive sites was calculated. PD and clinical attachment level (CAL) were measured in 0.5-mm increments using a University of North Carolina probe at six sites (buccal, mesio-buccal, disto-buccal, lingual, mesio-lingual, and disto-lingual) per tooth for all teeth. CAL was measured from the cemento-enamel junction to the bottom of the pocket. Periodontal indices were measured in the following order: GI, PLA, CAL, PD, and BOP. Teeth, gingiva, oral mucosa, and tongue were examined visually, even in the absence of adverse effects. The mean value of each parameter for each individual was used for statistical analyses.

General Examination

Weight, pulse, and blood pressure were measured upon completion of the oral examination. Blood tests and urinalyses were conducted by Japan Medical Laboratory. The following data were excluded from the blood analysis because of concerns regarding the influence of the meal: serum triglyceride levels within 6 hours after a meal and glucose levels within 4 hours after a meal. During the test period, all subjects were questioned regarding systemic and oral conditions to confirm the safety of the chewing gum.

Statistical Analysis

Data are expressed as a mean change from the baseline levels; furthermore, significance was analyzed with a repeated-measures analysis of variance (ANOVA) using statistical programs.‖¶ In the presence of interaction, significant differences among the means of the groups

‡ Red-Cote, Sunstar Americas, Chicago, IL.
§ Hu-Friedy, Chicago, IL.
‖ StatView, SAS Institute, Cary, NC.
¶ SPSS 10.0J, SPSS, Chicago, IL.

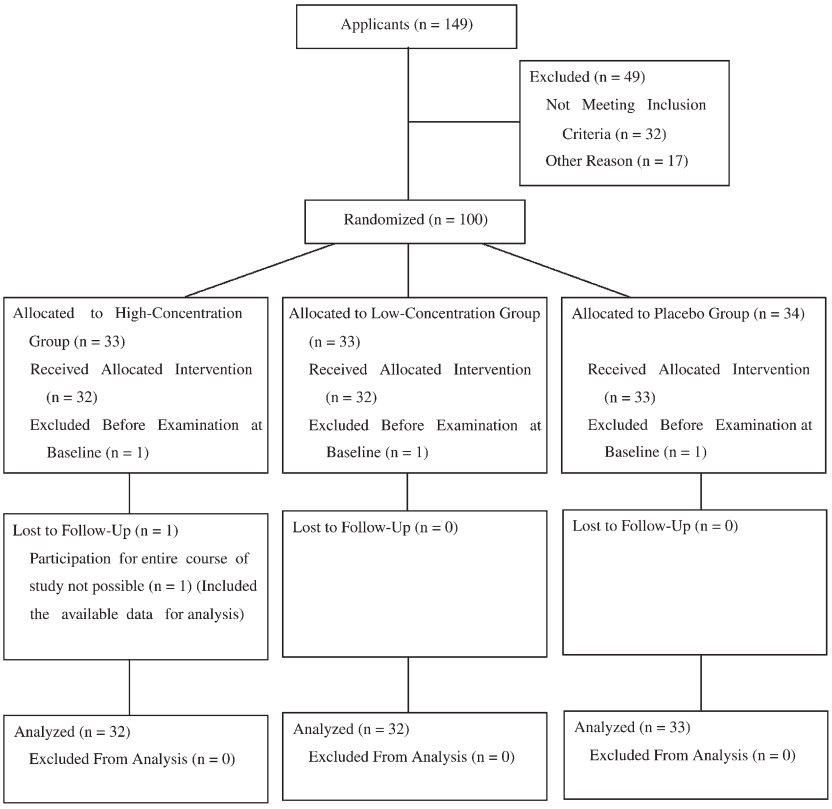

Figure 1.
Diagram illustrating the flow of participants through each stage of the randomized trial.

were determined by two-way ANOVA followed by the Games-Howell pairwise comparison test. The mean change at the designated time was compared to that at baseline within each group with the Dunnett test. The mean change at each point in each test group was also compared to that at the identical point in the placebo group with the Dunnett test.

RESULTS

Demographic Characteristics and Clinical Periodontal Parameters at Baseline

No statistical differences with respect to clinical periodontal parameters at baseline were observed among the groups (Table 2). A 4-week ration of test or placebo gum was distributed at baseline and at weeks 4 and 8; any unused gum was collected at the next examination. The gum use was calculated based on unused gum. The mean percentage of gum use was 96.6%, 99.4%, and 99.7% for the high-concentration, low-concentration, and placebo groups, respectively. Four subjects, two in the high-concentration group (69.5% and 77.6%), one in the low-concentration group (79.8%), and one in the placebo group (78.6%), consumed <80% of the assigned gum; however, all data were analyzed. Chewing time was confirmed by interviewing subjects at each examination.

Effectiveness of Eucalyptus Extract Chewing Gum on Periodontal Disease

A change in the mean of the periodontal parameter from that at baseline was used for analysis. Significant interactions among groups were observed with respect to PLA, GI, BOP, and PD by repeated-measures two-way ANOVA (P <0.05). As shown in Figures 2A through 2D, the low- and high-concentration groups demonstrated significant improvements in each of the aforementioned parameters compared to the placebo group by two-way ANOVA followed by the Games-Howell pairwise comparison test (P<0.05). No meaningful interaction was apparent for CAL (Fig. 2E). A comparison between the high- and low-concentration groups revealed significant differences in terms of PLA (P <0.05), whereas no statistically meaningful differences were evident for GI, BOP, PD, and CAL.

In cases of PLA, GI, BOP, and PD in which significant interactions were observed, the mean change at the designated time was compared to that at baseline within each group with the Dunnett test. Relative to that at baseline, meaningful decreases in PLA and GI were apparent at weeks 4, 8, 12, and 14 in the low- and high-concentration groups (P<0.01) but not in the placebo group (Table 2). In terms of BOP, significant declines were evident at weeks 4, 8, 12, and 14 in the low- and high-concentration groups (P <0.05) but not in the placebo group. PD was also improved markedly at weeks 4, 8, 12, and 14 (P<0.01) in the low-concentration group and at weeks 4 (P<0.05), 8, 12, and 14 (P <0.01) in the high-concentration group. Conversely, no significant effects were detected with respect to CAL.

Next, the mean change of the test group at each time point compared to that of the placebo group was analyzed by the Dunnett test. Mean changes in PLA, GI, and BOP in the low- and high-concentration groups displayed marked improvements at weeks 4, 8, 12, and 14 (P <0.05) compared to the placebo group (Table 2). Mean changes in PD in the high- and low-concentration groups were also significantly improved at each time point (P<0.05) relative to the placebo group, with the exception of week 4 in the low-concentration group. Significant differences in CAL were observed at weeks 8, 12, and 14 in the low- and high-concentration groups compared to the placebo group (P<0.05).

Table 2.

Effect of Eucalyptus Extract Chewing Gum on Periodontal Parameters (mean ± SD)

Parameter	Group	Baseline	4 Weeks (change)*	8 Weeks (change)*	12 Weeks (change)*	14 Weeks (change)*
PLA (mm)	TH	1.86 ± 0.66	−0.43 ± 0.34†‡	−0.47 ± 0.37†‡	−0.49 ± 0.45†‡	−0.43 ± 0.33†‡
	TL	1.90 ± 0.62	−0.26 ± 0.31†‡	−0.30 ± 0.40†‡	−0.38 ± 0.34†‡	−0.35 ± 0.37†‡
	C	1.69 ± 0.80	−0.02 ± 0.35	0.01 ± 0.31	0.06 ± 0.44	0.15 ± 0.41
GI	TH	0.83 ± 0.31	−0.24 ± 0.25†‡	−0.29 ± 0.24†‡	−0.36 ± 0.29†‡	−0.29 ± 0.29†‡
	TL	0.85 ± 0.36	−0.19 ± 0.22†‡	−0.25 ± 0.26†‡	−0.28 ± 0.29†‡	−0.25 ± 0.29†‡
	C	0.80 ± 0.34	−0.03 ± 0.21	−0.02 ± 0.26	−0.05 ± 0.24	−0.01 ± 0.24
BOP (%)	TH	48.54 ± 25.55	−11.24 ± 11.30‡§	−17.84 ± 16.57‡§	−19.71 ± 15.64‡§	−19.01 ± 16.46‡§
	TL	42.86 ± 22.80	−9.16 ± 13.24‡§	−13.98 ± 17.75‡§	−14.66 ± 19.72‡§	−14.05 ± 16.11‡§
	C	38.57 ± 26.25	−0.57 ± 10.03	3.26 ± 15.39	1.71 ± 13.82	4.02 ± 16.01
PD (mm)	TH	2.20 ± 0.25	−0.06 ± 0.12‡§	−0.10 ± 0.16†‡	−0.10 ± 0.09†‡	−0.11 ± 0.11†‡
	TL	2.24 ± 0.23	−0.06 ± 0.10†	−0.07 ± 0.09†‡	−0.07 ± 0.09†‡	−0.12 ± 0.14†‡
	C	2.21 ± 0.23	−0.02 ± 0.14	+0.02 ± 0.14	0.02 ± 0.09	0.02 ± 0.17
CAL (mm)	TH	2.25 ± 0.40	−0.03 ± 0.19	−0.07 ± 0.20‡	−0.07 ± 0.18‡	−0.07 ± 0.19‡
	TL	2.26 ± 0.45	−0.03 ± 0.21	−0.05 ± 0.21‡	−0.05 ± 0.21‡	−0.08 ± 0.24‡
	C	2.26 ± 0.40	−0.01 ± 0.16	0.03 ± 0.20	0.03 ± 0.20	0.03 ± 0.23

TH = high-concentration group; TL = low-concentration group; C = placebo group.
* A negative value indicates improvement.
† Statistical significance between baseline and observed time point with the Dunnett test ($P<0.01$).
‡ Statistical significance compared to the placebo group with the Dunnett test ($P<0.05$).
§ Statistical significance between baseline and observed time point with the Dunnett test ($P<0.05$).

During the course of this study, abnormal effects on teeth, gingiva, oral mucosa, and tongue were not observed on visual examination or with detailed questioning of subjects regarding their oral conditions; one participant in the high-concentration group complained of staining on her teeth. However, the staining was very slight, and the possibility that it was caused by the eucalyptus chewing gum was extremely low.

Adverse Events

Four subjects (one in the high-concentration group, two in the low-concentration group, and one in the placebo group) complained of transient diarrhea; however, no other serious general symptoms were observed. During the test period, no critical changes were detected in weight, pulse, or blood pressure (data not shown). Blood test results were within normal limits for all subjects during the test period (data not shown). Urinalysis results during the test period were unremarkable with two exceptions: one individual in the high-concentration group displayed a positive glucose reading at baseline, and a subject in the placebo group demonstrated occult blood at week 14.

DISCUSSION

The primary objective of this study was to evaluate the effect of eucalyptus extract chewing gum on periodontal health, especially gingivitis. Interaction between the effects of eucalyptus extract chewing gum and the intake period was significant for PLA, GI, BOP, and PD using repeated-measures two-way ANOVA; moreover, with respect to these parameters, meaningful improvement was demonstrated in the test groups relative to the placebo group by the Games-Howell pairwise comparison test. These results indicated that eucalyptus extract chewing gum might be beneficial in terms of improvement of periodontal health in cases involving gingivitis. The effectiveness of eucalyptus extract chewing gum on periodontal health was also confirmed based on the following results: statistically significant improvements in PLA, GI, BOP, and PD were obtained after 4 weeks relative to baseline in the test groups, and, even at 14 weeks (2 weeks after discontinuing use of the gum), significant improvement was retained in each of the aforementioned parameters. As shown in Table 2, mean PLA and BOP at baseline in the test groups were slightly greater than in the placebo group, despite the lack of statistical significance. However, mean PLA and BOP at 14 weeks in the test groups were significantly lower than in the placebo group (data not shown); moreover, PLA and BOP of the test groups, which were markedly improved over time, were statistically significantly better compared to those values in the placebo group at each time point (Table 2). This finding indicated that

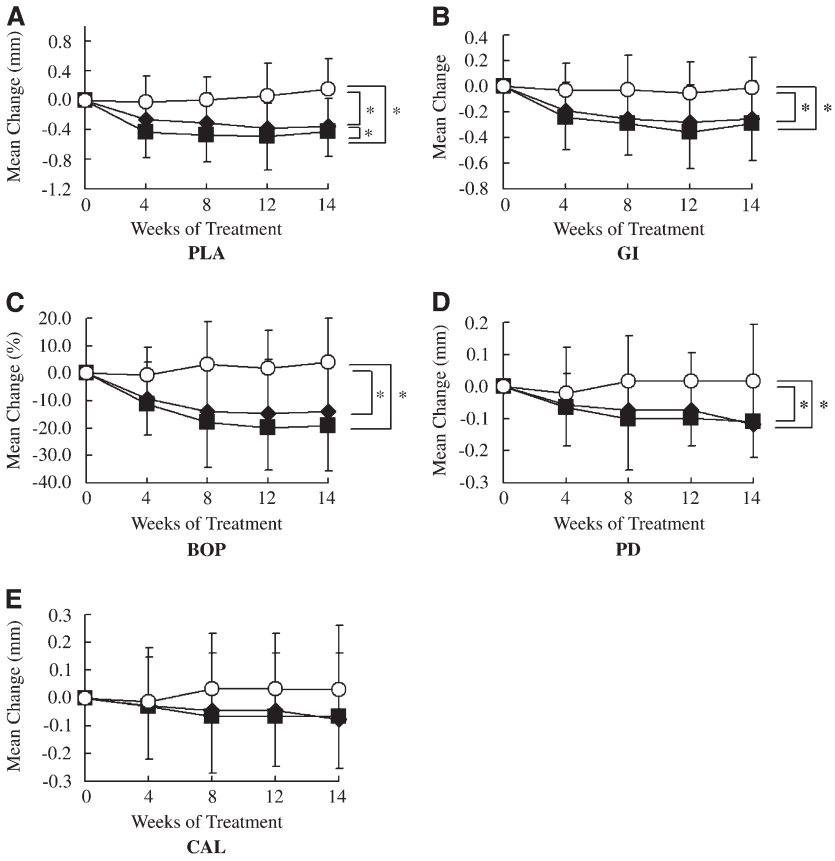

Figure 2.

*Effect of eucalyptus extract chewing gum on clinical signs of periodontal disease. PLA **(A)**, GI **(B)**, BOP **(C)**, PD **(D)**, and CAL **(E)** were measured at baseline and at 4, 8, 12, and 14 weeks after baseline. The data represent the mean change ± SD from the values at baseline. Solid squares = high-concentration group; solid diamonds = low-concentration group; open circles = placebo group. *Significant differences among the groups (P <0.05 as determined by repeated-measures two-way ANOVA followed by the Games-Howell pairwise comparison test).*

eucalyptus extract chewing gum might contribute to improvement in PLA and BOP.

Among eucalyptus extracts, macrocarpals, including macrocarpal C, are the main components possessing antibacterial activity against cariogenic and periodontopathic bacteria;[4,5] however, in the absence of macrocarpal purification, chewing gum containing 60% ethanol extracts of *E. globulus* leaf was considered sufficiently effective for gingivitis. One possible explanation why eucalyptus extract chewing gum ameliorates gingivitis pertains to its antibacterial activity against periodontopathic bacteria; eucalyptus extracts inhibited the growth of periodontopathic bacteria[7] and affected virulence factors of *P. gingivalis*.[5] However, given that the antibacterial activity of eucalyptus extracts against *P. gingivalis* and *P. intermedia* was bacteriostatic and not bactericidal,[7] the primary reason for improvement of gingivitis in the presence of eucalyptus extracts may be attributable to inhibition of the growth of various oral bacteria, including Gram-positive and -negative bacteria, resulting in decreased accumulation of dental plaque. PLA decreased in the test groups; furthermore, the decrease in the high-concentration group was significantly greater than that in the low-concentration group. Takahashi et al.[6] noted that the numbers of *S. mutans* on the teeth of germ-free BALB/cA mice receiving feed containing extract of *E. globulus* were substantially lower than those of the control group. Moreover, Sato et al.[18] indicated that eucalyptus extract chewing gum led to a significant reduction in plaque formation compared to the control gum in a preliminary study. Therefore, it is reasonable to conclude that improvements in GI, BOP, and PD might occur as a result of the inhibition of plaque accumulation by eucalyptus extract chewing gum, even at a low concentration. In terms of PLA, a significant difference was detected between the high- and low-concentration groups, which was indicative of dose-response effects; however, no significant dose-response effects were observed for other periodontal parameters. Additional investigations are necessary to clarify the effect of eucalyptus extracts on bacterial flora in vivo and the dose-response effects; however, to the best of our knowledge, this article is the first to demonstrate the effectiveness of eucalyptus extract chewing gum with respect to periodontal health.

Approximately 30 subjects were selected for each group in this investigation. Although the sample size was not calculated in advance, power analysis (α = 0.05; power = 0.08) revealed that the sample sizes for PLA, GI, BOP, PD, and CAL were 15, 24, 18, 25, and 76, respectively. With the exception of CAL, the sample size used in this study was sufficient. Sample size might affect the result obtained for CAL, for which no meaningful difference was evident. Three subjects were excluded prior to the baseline examination; additionally, one subject who underwent the baseline examination was lost before the 4-week examination. All available data, including his data at baseline, were analyzed to prevent unknown bias, which might have been introduced by the subject's premature termination.

Eucalyptus extracts have been used for diverse purposes, including as a food source, for many centuries; furthermore, these extracts are believed to be safe. Neither abnormal oral nor general adverse effects were observed in the present study. Transient

diarrhea was evident in four participants. The gum used in this investigation included sugar alcohol, e.g., xylitol and maltitol. In tests involving the administration of a single dose, the 50% laxative effective dose of xylitol was estimated as 0.52 g/kg for males and 0.70 g/kg for females,[19] whereas that of maltitol was estimated as 0.8 g/kg for males and females.[20] The maximum non-effective dose of xylitol was estimated as 0.37 g/kg for males and 0.42 g/kg for females,[21] and that of maltitol was estimated as 0.3 g/kg for males and females.[20] The amounts of xylitol and maltitol contained in the chewing gum used in this study were 6.66 g/day and 5.08 to 5.11 g/day, respectively. In terms of human body weight, the risk that these sugar alcohols would induce diarrhea seemed to be very low.

Rats received feed containing 0.5% eucalyptus extract (mean amounts of eucalyptus extract intake per day were 310 mg/kg for male rats and 350 mg/kg for female rats) for 13 weeks. In the test groups, toxicity was not observed in the general condition, clinical and biochemical tests (blood test and urinalysis), or upon pathologic observation (unpublished data). The quantities of eucalyptus extracts in this study were 90 mg/day in the high-concentration group and 60 mg/day in the low-concentration group. Based on the assumption of 60-kg body weight, the amounts of eucalyptus extract per kilogram were 1.5 mg/day in the high-concentration group and 1.0 mg/day in the low-concentration group, which were equivalent to 1/200 and 1/300, respectively, of the non-effective dose of eucalyptus extracts in the rat experiments. Approximately 24 g eucalyptus extract was obtained from 100 g *E. globulus* leaf; thus, 90 mg eucalyptus extract was equivalent to ~375 mg *E. globulus* leaves.

CONCLUSIONS

Eucalyptus extract chewing gum had a meaningful effect on plaque, gingivitis, and BOP in human clinical trials. In terms of the safety and suitability of the daily use of eucalyptus extract chewing gum, this study provided evidence for its potential use as a new functional food for periodontal health distinct from toothpaste and mouthwash.

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