치의학석사 학위논문

The effect of new dentifrices containing Centella asiatica and Bamboo salt on reducing plaque and gingivitis

- a randomized clinical trial -

치은염과 치면세균막에 대한
Centella asiatica 및 죽염 함유 세치제의 효과분석
- 무작위 임상 실험 결과를 중심으로-

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서울대학교 대학원
치의과학과 예방 및 사회치의학 전공
김 미 선

Abstract

The effect of new dentifrices containing Centella asiatica and Bamboo salt on reducing plaque and gingivitis

- a randomized clinical trial -

Mi-Sun Kim, RDH, BA

Department of Preventive and Social Dentistry,

The Graduate School, Seoul National University

(Directed by Professor **Hyun-Duck Kim, DDS, MSD, phD**)

Objective: This study aims to evaluate the effect of dentifrice formulations containing Centella asiatica (CA) and bamboo salt (BS) on gingivitis and dental plaque.

Methods: A 4-day randomized crossover clinical trial with 10-day washout period was applied for two experimental dentifrices and a standard-control dentifrice (Control). Thirty-three volunteers with the mean age of 28 years were randomized into 3 groups. One experimental dentifrices contained Control plus CA (DCA) and the second experimental dentifrice contained an additional component of BS (DCA-BS). Each participant used the assigned

dentifrices to brush their teeth for 3 minutes twice a day. Pre- and post-experiment clinical examinations of the upper teeth were performed. The gingival index (GI) was assessed using CPI probe at 60 gingival sulcus sites among 10 teeth. Turesky-modified plaque index (PI) was assessed in 30 tooth surfaces of 10 teeth. The difference between pre- and post-experimental data was analyzed using Wilcoxon signed-rank test for GI and paired t-test for PI. Wilcoxon signed-rank test was also used to compare treatment efficacy.

Results: DCA-BS reduced GI by 72% after experiment. Compared to Control, the GI reduction ratio of DCA-BS was 3.3 times. However, there was no difference in the effect on PI among DCA-BS, DCA and Control. Our data showed that the new dentifrices containing CA and BS could reduce the risk of gingivitis. Further study is indicated.

Conclusions: The dentifrices containing CA and BS could be used to promote gingival health in young adults in both home care and clinical application.

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keywords : dentifrice, Centella asiatica, bamboo salt, gingivitis, randomized clinical trial

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Ⅰ. Introduction

Gingivitis is an inflammatory condition of the supporting structures around the teeth that arise as the result of the interactions among microorganisms residing inthe dental plaque [1]. Plaque control is the most widely used and effective means of controlling gingivitis [2]. Self-performed mechanical plaque control, in particular, is a universal practice for controlling plaque and gingivitis [3, 4]. However, most adults usually cannot remove plaque completely from all tooth surfaces [5]. Therefore, various types of agents have been added to toothpaste to improve the effectiveness of plaque removal and hence, prevent gingivitis [6, 7]. A number of agents have been evaluated for their efficacy against gingival disease and dental caries, such as chlorhexidine [8, 9], Listerine [9-11], triclosan [12] and olive oil [13].

With growing interest in plant-based products among researchers, many toothpastes with various herbal extracts have been introduced over the past few decades [14-16]. Centella asiatica (CA), a small herb broadly cultivated in China, Southeast Asia, India and Oceanic countries, has long been used for therapeutic purposes since ancient times [17]. CA has been reported to be useful in the treatment of skin diseases [18] wound repair [19] and healing of burn [17]. In dentistry, few studies have explored the efficacy of CA. In one study, CA was demonstrated to be an effective supportive agent following periodontal treatment, resulting in reduced plaque and gingivitis [20]. In spite of such wound healing properties of CA, its usage in dental field has been limited.

Bamboo salt (BS) is a Korean folk remedy consisting of sea salt roasted in bamboo which has long been used for therapeutic purposes in Korea [21]. Toothpastes containing BS has been sold in Korea for some decades. Some in vitro studies [21-23] and animal studies using rats [24-26] demonstrated

anti-inflammatory effects of BS. Nevertheless, no human study has yet evaluated the effect of BS on periodontal health.

Therefore, the new dentifrice containing CA and BS was developed. We made a hypothesis that dentifrices containing CA and BS could surpass the effect of standard sodium fluoride dentifrice on the reduction of dental plaque and gingivitis. The aim of this study was to evaluate the efficacy of the new dentifrice containing CA and BS in the reduction of dental plaque and gingivitis among participants without established periodontitis.

Ⅱ. Materials and Methods

Ethical issue and study design

Prior to this clinical trial, ethical approval was obtained from the Institutional Review Board of Seoul National University Dental Hospital (approval number: CRI 09001). A written informed consent was obtained from all participants in the study. All participants had the right to abstain from the trial at any time during the entire period of the trial. This study was designed as a randomized, triple-blinded (designer, evaluator and participants) clinical trial with factorial design [27].

Inclusion and exclusion criteria

All participants were healthy Korean adults who volunteered to participate in this study through advertising posters. The inclusion criteria included the participants who are 20-40 years old, those with no systemic disease and

those with at least 20 remaining teeth (excluding third molars). Participants were excluded from the study if they had received antibiotics within 3 months, had deep periodontal pockets ($\geq$5mm), had dentures or clinically unacceptable restorations or bridges or orthodontic appliances, or are pregnant or lactating women.

Sample size calculation

According to the data from a pilot study, the difference in gingivitis index (GI) scores by modified Sulcus Bleeding index [28] between pre- and post-experiment was 1.1 (0.5 for pre-experiment and 1.6 for post-experiment) with the standard deviation of 2.2. Under the condition of type I error at 0.05 and type II error at 0.2, the number of subjects needed for Wilcoxon singed-rank test was estimated at 32. For equal allocation into three groups (11 participants per each group), we decided the samples size as 33.

Recruitment and randomization

At the first appointment for recruitment, the purpose and risks of this investigation were fully explained to all participants. After that, all participants provided the written informed consent. A total of 33 volunteers, 18 males and 15 females, were randomized into three identical groups (Table 1). For randomization, according to the information from the examination at the time of recruitment by one examiner, the participants were stratified into three balanced groups by the designer. The information was based on age, gender, DMFT index by WHO criteria, Turesky-modified Quigley-Hein plaque index (PI) [28, 29], Löe-Silness gingival index (GI) [28] and smoking. In this randomized blinded clinical trial, Latin square allocation was applied for reducing the carryover problems inherent in

cyclical permutation designs. Age, gender, DMFT, PI and GI were not different across three groups (Table 2). An appropriate randomization was accomplished in this trial.

Triple blinded trials and Cross-over

The experimental process was carried out in triple-blinded manner for participants, a calibrated examiner (MS Kim) and the designer (HD Kim) by masking the labels of three types of dentifrices until the end of the final analysis. All experimental dentifrices were filled in plain white tubes, labeled "A", "B" and "C" by the supplier, LG Household & Healthcare Co., Seoul, Korea. Control contained dental-type silica, sodium fluoride and aminocaproic acid. One of the experimental dentifrices contained Control ingredients plus CA titrated extract (DCA), the second experimental dentifrice contained an additional component of bamboo salt (DCA-BS).

Following each 4-day trial, there was a ten-day washout period to eliminate the carry-over effects of the previous trial. During the entire clinical trial period, smoking and alcohol consumption were not allowed.

Each participant brushed their teeth using only the assigned dentifrices for 3 minutes twice a day (after breakfast and before bedtime). All participants used the assigned dentifrices for 4 days in each trial cycle Control was used during the 10-day washout period. To remove the effect of different toothbrushes, a soft-bristled toothbrush was given per a cycle. On the first day of each clinical cycle and after the four-day trial period, clinical examinations were carried out to evaluate the pre and post experimental GI, PI scores and any side effects (Table 1).

Plaque score collection

After taking three photos (frontal and two laterals) using a digital camera (OLYMPUS SP-565UZ), all 10 upper teeth except molars were selected to evaluate dental plaque per person. Prior to taking photos, participants were recommended to gargle 15ml of distilled water containing four drops of 1% of erythrosine red solution for one minute for discoloration of teeth surface with plaque. Turesky-modified Quigley-Hein plaque index [29], was applied to evaluate plaque in labial surfaces. The labial surface of tooth was divided into three parts and the plaque score per part graded on 0-5 scale (0=normal, 1= dot plaque, 2= line plaque, 3= less than 1/3 plaque, 4= less than 2/3 plaque, 5=2/3 plaque or more).

Gingival index collection

All 10 upper teeth except molars were selected per person. GI was scored within 10 secondsafter probing at mesial, central and distal gingival sulcus sites (six sites) per tooth using CPI manual probe and graded on a 0-2 scale (0=normal, 1=dot bleeding, 2=line [1>mm] bleeding). Hence, 60 sites were evaluated per each person.

Statistical analysis

Age, plaque index and gingival index had highly skewed distribution (Kolmogrov-Smirnov, $p<0.05$), and hence non-parametric Kruskal-Wallis test was applied but mean values were shown instead of median for easier understanding. DMFT index was normally distributed (Kolmogrov-Smirnov, $p>0.05$) and hence, analysis of variance (ANOVA) was applied. The difference in the sum of GI scores (SOG) between pre- and post-

experiment were not normally distributed (Kolmogrov-Smirnov, $p<0.05$) and hence, Wilcoxon signed-rank test was used to evaluate the effectiveness of the toothpaste on gingivitis. Paired t-test was applied to evaluate the change in the sum of PI score (SOP), the data of which was normally distributed (Kolmogrov-Smirnov, $p>0.05$). Because SOG and SOP of all groups were different at the baseline and some of them had the value of null, the modified Johnson-Neyman procedure [7] was applied to estimate the reduction rate of SOG and SOP within group. Reducion rate was defined as follows.

For evaluating the difference in reduction rate between the dentifrices on SOG and POG, Wilcoxon signed-rank test was applied.

Ⅲ. Results

There were no differences among the three allocated groups in terms of age, gender, smoking history and DMFT, plaque and gingival indices (Table 2). No adverse reactions were reported during the entire experimental procedure. The reliability for PI score was high with the intraclass correlation coefficient of 0.972.

SOG of post-experiment decreased compared to that of pre-experiment for all dentifrices (Fig. 1): from1.73 to 0.45 for DCA-BS, from 1.45 to 0.61 for DCA, from 1.94 to 1.52 for Control. However, the difference was statistically significant only in DCA-BS group ($p=0.025$).

SOP of DCA-BS and control was reduced after the experiment: from 37.50 to 33.16 for DCA-BS, from 37.90 to 33.52 for Control. However, SOP of DCA increased ‐8 from 31.78 to 38.81. However, all SOPs were not

statistical different between pre- and post-experiment across three dentifrices.
The reduction rate of SOG was highest in DCA-BS among three dentifrices: 72% for DCA-BS, 60% for DCA, 22% for Control (Table 2). There was statistically significant difference (p=0.038) between DCA-BS and the control. Compared to Control, the SOP reduction ratio of DCA-BS was 3.3 times.
The reduction rate of SOP was similar in DCA-BS (11%) and Control (12%)while DCA showed an increase. However, all of these SOP were not statistically different (p>0.05) across groups.

Ⅳ. Discussion

Our data showed that dentifrice containing both CA and BS can reduce gingivitis among young adults. The application of CA alone was not effective and CA and BS did not reduce plaque. To the best of our knowledge, our data is the first evidence that evaluated the effect of CA and BS on gingivitis and dental plaque by randomized controlled trial.
Our data has some strengths. First, we adopted triple-blinded procedure for the designer, examiner and the participants. Second, Latin square design was constructed to minimize the carryover problems inherent in cyclical permutation designs. Fleiss [30]describes a simple method which produces a useful balanced carryover Latin square design. Latin square design made carryover effects suitably balanced for 7-h bacterial count study [31]and 4-day plaque regrowth study [32]. Third, our participants were healthy young adults who are more likely to be medically fit with less confounding

factors.

According to our data, BS and DCA, when combined, reduced gingivitis. When DCA was used alone, there was a tendency to reduce gingivitis which was, nonetheless, not statistically significant. However, neither BS nor DCA had an effect on dental plaque reduction. This implies that the reduction mechanism of gingivitis may not be associated with dental plaque reduction.

Previous studies showed that CA promotes wound healing [17-19]. CA was reported to have pharmacological properties such as promoting fibroblast proliferation and stimulating collagen synthesis [33]. An animal study on rats showed that CA had anti-oxidant effect and reduced experimental arthritis in rats [34]. A clinical trial showed that adjunctive local delivery of CA and P. granatum extracts significantly improved clinical signs of chronic periodontitis and reduced IL-1â level in periodontal maintenance patients [20]. Some dentifrices with herbal extraction other than CA have been associated with reduction of gingivitis and dental plaque [14, 35]. On the other hand, a randomized clinical trial with dental students showed that an herbal toothpaste reduced gingivitis while reduction dental plaque was not different from control [16]. Another clinical trial showed that the use of herbal toothpaste did not result in greater reduction of gingivitis or dental plaque than the control [15].Further study is indicated to confirm our findings.

For a randomized clinical trial, our data is the first to report the effect of BS on promoting periodontal health. BS has been associated with anti-inflammation in several animal studies and in vitro studies [21-26]. The reduction of gingivitis by BS could be due to its anti-inflammatory effect. An in vitro study showed that BS increased lipid membrane fluidity more than NaCl salt due to lipid-substrate and lipid-salt interaction [36]. However, our data showed that neither BS nor CA play a role in preventing dental

plaque accumulation. Further studies are indicated to elucidate the mechanism.

Our study has some limitations. First, the cross-over design of the trial inevitably involves carry-over effects which means that the effect of the previous experiment may impose influence on the following experiment. Hence, we had washout periods of 10 days in-between the experiments and performed professional tooth cleaning at the end of each experiment. The trial period of 4 days could be short to evaluate the effectiveness of each dentifrice. We used the 4-day brushing twice-a-day model instead of the 4-day plaque re-growth model [37]. Although the 4-day plaque re-growth model would allow better identification of differences than the 4-day brushing model, we decided to apply the 4-day brushing model based on ethical and practical considerations. Notwithstanding these limitations, our data is valid enough to suggest the efficiency of new dentifrice containing CA and BS on reduction of gingivitis.

Ⅴ. Conclusion

Our randomized controlled trial showed that the dentifrice containing CA and BS could reduce gingivitis while plaque reduction was not different from control. Further studies are needed to confirm our results and the mechanism.

Table 1. Four-day randomized controlled trial with cross-over design and examination procedure.

Contents	Start	WO	Cycle 1	WO	Cycle 2	WO	Cycle3	Recovery	Final
Date	D1		D11 D15		D25 D29		D39 D43		D50
Period (days)	1	10	4	10	4	10	4	7	1
Latin Square Allocation									
Group 1 (n=11)			Trial A		Trial B		Trial C		
Group 2 (n=11)			Trial B		Trial C		Trial A		
Group 3 (n=11)			Trial C		Trial A		Trial B		
<Check lists>									
Dental status	√								√
Personal data	√								
Medical history	√								√
Plaque index	√		√ √		√ √		√ √		√
Gingival index	√		√ √		√ √		√ √		√
Side effect	√		√ √		√ √		√ √		√
Professional tooth cleaning	√		√		√		√		√

WO denotes washout period.

Table 2. Characteristics of the three allocated groups.

Variable	Category	Group 1 (n=11)	Group 2 (n=11)	Group 3 (n=11)	p-value
Gender, n(%)	Male	6(54.5)	6(54.5)	6(54.5)	1.000[a]
	Female	5(45.5)	5(45.5)	5(45.5)	
Age (year), mean+SD		28.18±7.43	28.27±3.55	27.91±3.99	1.000[b]
Smoking, n(%)	Yes	1(9.1)	1(9.1)	1(9.1)	1.000[a]
	No	10(90.9)	10(90.9)	10(90.9)	
DMFT index, mean+SD		5.09±4.32	6.73±5.08	7.55±3.27	0.404[c]
Plaque index[d], mean+SD		0.91±0.83	0.45±0.52	0.45±0.52	1.000[b]
Gingival index[e], mean+SD		2.73±5.10	1.27±2.05	3.55±4.32	1.000[b]

SD denotes standard deviation.

a p-values obtained from chi-square test.

b p-value obtained from Kruskal-Wallis test.

c p-value obtained from one-way ANOVA.

d Plaque index denotes the sum of plaque index scores obtained from the 30examined surfaces in 10 upper teeth.

e Gingival index denotes the sum of gingival index scores obtained from 60gingival sulcus sites in 10 upper teeth.

Table 3. Comparison of the dentifrices on the reduction rate of gingivitis and plaque in upper dental arch within the same participants (n=33).

Variable	Group	Reduction rate[c]	P-valued	
		Mean (SD)	Control	DCA[b]
Gingival index	DCA-BS[a]	0.72 (1.94)	0.038	0.797
	DCA[b]	0.60 (2.24)	0.128	-
	Control	0.22 (3.46)	-	-
Plaque index	DCA-BS[a]	0.11 (0.48)	0.879	0.127
	DCA[b]	-0.21 (1.21)	0.270	-
	Control	0.12 (0.55)	-	-

Bold denotes statistical significance at $p<0.05$.

SD denotes the standard deviation.

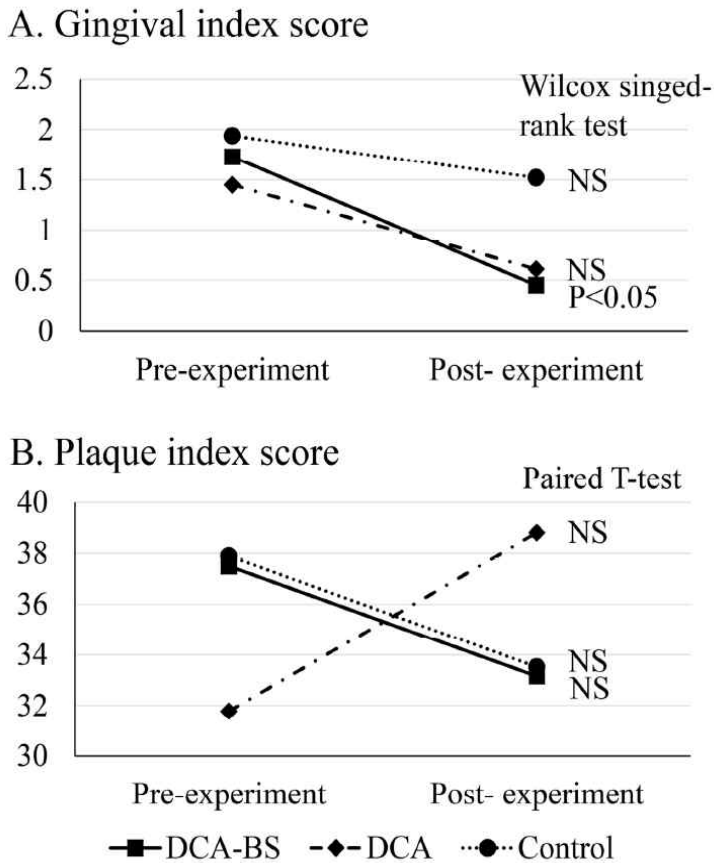
a DCA-BS denotes dentifrice with Centella asiatica , bamboo salt and control ingredients.

b DCA denotes dentifrice with Centella asiaticaand control ingredients.

c Reduction rate: (pre-experiment SOG – post-experiment SOG) / pre-experiment SOG.

d P-values obtained from Wilcoxon signed-rank test. Pairs of two groups were compared.

Fig. 1. Change of gingivitis (gingival index) and supragingival plaque across dentifrices over experimental period (n=33).



Note : Gingival and plaque index scores are the sum of scores in examined teeth. DCA-BS denotes dentifrice with Centella asiatica, bamboo salt and control ingredients, and DCA denotes dentifrice with Centella asiatica and control ingredients. NS denotes statistically non-significant at $p>0.05$.

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국문초록

치은염과 치면세균막에 대한 Centella asiatica 및 죽염 함유 세치제의 효과분석 - 무작위 임상 실험 결과를 중심으로-

서울대학교 대학원 치의과학과 예방 및 사회치의학 전공
(지도교수: 김현덕)

김미선

1. 목 적 : 본 연구에서는 치은염과 치면세균막을 가진 피험자를 대상으로 Centella asiatica(CA) 및 죽염(BS: Bamboo salt)이 함유된 세치제의 효과를 평가하였다.

2. 방 법 : 본 연구의 임상실험은 10일간의 휴약기(washout)를 포함한 14일간의 무작위 추출 교차 실험으로 설계 후 진행되었다. 평균 28세 연령인 33명의 피험자는 3그룹으로 무작위 배정되었고, 실험 세치제는 대조군 세치제에 CA를 포함한 세치제(DCA)와 이에 더하여 죽염까지 포함시킨 또 다른 실험 세치제(DCA-BS)의 두 종류로 구성하였다. 각각의 참가자들은 하루에 2회 3분간 할당된 세치제를 사용하여 잇솔질 하였고, 잇솔질 전 후에 임상검사를 시행하였다. 치은염지수(GI)는 CPI probe를 이용하여 10개의 치아 60곳의 치은열구에서 측정하였고, 치면세균막 지수(PI)는 10개 치아 30개의 면에서 Tureskey-modified plaque index를 이용하여 측정하였다. GI의 실험

전후 차이를 분석하기 위해 Wilcoxon signed-rank test를 시행하였으며, PI의 실험 전후 차이를 확인하기 위해서는 paired t-test를 이용하였다.

3. 결 과 : 그 결과 DCA-BS는 실험 후 72%의 치은염 감소를 보였고, 대조군 대비 3.3배의 감소율을 보였다. 그러나 DCA-BS 및 DCA와 대조군 사이의 치면세균막 감소의 효과는 통계적으로 유의한 차이를 보이지 않았으므로, 향후 후속연구가 필요할 것으로 보았다. 결론적으로 우리의 연구는 CA와 BS가 포함된 새로운 세치제에서 치은염 감소효과가 나타날 수 있음을 확인 하였다.

4. 결 론 : CA와 BS가 포함된 세치제를 사용하는 것은 가정과 임상에서 구강건강 관리 시 젊은 연령층의 치은 건강 증진에 유용할 것으로 기대된다.

주요어 : 세치제, Centella asiatica(CA), 죽염, 교차실험, 무작위 임상실험
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