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ORIGINAL ARTICLE

Clinical and antibacterial effect of an anti-inflammatory toothpaste formulation with *Scutellaria baicalensis* extract on experimental gingivitis

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Abstract It was the aim of the study to evaluate the clinical and antibacterial effect of a dentifrice containing an anti-inflammatory plant extract (SB) versus a placebo (PLA) using an experimental gingivitis model. Forty subjects (20 per group) discontinued all oral hygiene measures for four teeth for a period of 21 days using a shield (to generate a possible gingivitis) while they could brush the other teeth normally. After brushing, the shield was removed and teeth were treated with the randomly assigned toothpaste slurry for 1 min. Löe and Silness gingival index (GI), Silness and Löe plaque index (PI), and biofilm vitality (VF%) were assessed at days 0, 14, and 21, respectively. Subjects of the PLA group developed a GI of 0.82±0.342 (day 14) and 1.585±0.218 (day 21), while the data of the SB group were significantly reduced (0.355±0.243 and 0.934±0.342, $p<$ 0.001). While PI was significantly reduced at all follow-up appointments, reductions in VF reached the level of significance only at day 21. The results suggest that the new toothpaste formulation was able to significantly reduce the extent of gingivitis, plaque development, and vital flora.

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Introduction

Adding active agents to dentifrices or mouthrinses is a common method to support mechanical plaque removal and to provide a preventive effect against caries and gingivitis. The most important group of therapeutic agents in gingivitis prevention are antibacterial agents. However, since gingivitis and periodontitis are characterized by inflammatory symptoms, the use of anti-inflammatory agents such as NSAIDs like flurbiprofen, naproxen, and ibuprofen have potential therapeutic value [1–6]. Criticisms are derived from the systemic side effects for the systemically administered NSAIDs and especially for locally applied agents (e.g., dexiprofen mouth rinse; [7]) due to the fact that they potentially mask inflammation while they do not treat the cause, i.e., biofilm [6]. An ideal agent would combine antibacterial and anti-inflammatory properties.

In search of new agents especially for herbal products, a plant extract was found which exerted anti-inflammatory and some antibacterial properties, *Scutellaria baicalensis* Georgi (Chinese skullcap). The plant was used extensively as a component of traditional herbal (Chinese) medicines and also as a dietary ingredient, mainly in China, Japan, Korea, and the Russian Federation, as well as in the United States. It has a well-documented history of use spanning 2,000 years. The roots of the plant contain a variety of bioactive chemical constituents, including the flavonoids baicalein, baicalin, wogonin, and acteoside, which have

shown significant free radical scavenging and antioxidant activities, among other beneficial effects, in numerous pharmacological studies in vitro and in vivo. The roots of *S. baicalensis* or their flavonoid components have been associated with a variety of pharmacological actions, including antimicrobial, antiallergic, anti-inflammatory, diuretic, cholagogic, choleretic, spasmolytic, antihypertensive, and antipyretic actions, as well as effects on the central nervous system and lipid metabolism, e.g., [8, 9]. Due to its significant antioxidant capacity, recent studies have suggested that it could serve as an antioxidant food ingredient similar to other common flavonoid-containing foods, such as green tea, fruits, vegetables, and beans [10]. The property of herbal extracts in inhibition of COX-2 is so far a suitable approach for the prevention and therapy of cancer [11, 12]. Newer findings showed some benefits of *S. baicalensis* in adjuvant therapy of prostate cancer [13, 14], hepatocellular carcinoma [15], and for the prevention of oral cancer [16].

All these positive properties lead to the hypothesis that this agent could act as a sensible adjuvant in oral hygiene products which are aimed to prevent and possibly treat gingival inflammation by daily use in a dentifrice.

It was the aim of the study to evaluate the clinical effect of a new dentifrice formulation with *S. baicalensis* extract (0.5%) during the course of 21 days using an experimental gingivitis model.

Material and methods

The study was a double-blind, prospective, randomized, intra-individual comparative, single-center clinical study. Participants were placed in parallel groups, and the study was performed in the Department of Operative Dentistry and Periodontology, Dental School and Hospital, Freiburg, Germany.

The protocol for the study was reviewed and approved by the Medical Ethical Committee of the University of Freiburg and not commenced until approval had been obtained (No. 173/05).

The study was conducted and monitored in accordance with the ICH note for guidance on Good Clinical Practice and the Helsinki declaration. Prior to participation, the purpose and risks of the investigation were fully explained to all participants. Subjects were entered into the study only after having given written consent.

Study population

A total of 40 (24 female and 16 male) subjects with a mean age 25.05±3.63 years participated in this study. Inclusion criteria were a minimum of four natural uncrowned teeth (excluding 3rd molars) in one quadrant present, good general health, and had given written informed consent. Subjects were excluded from the study if they were pregnant or breastfeeding, if there was evidence of antibiotic use during the 4 weeks prior to the study, if the patients had to take anticoagulants, or if they were allergic to personal care/consumer products or their ingredients, relevant to any ingredient in the test products as determined by the dental/medical professional monitoring the study.

Test products

The dentifrices to be used for the groups were a control toothpaste with fluoride (1,400 ppm stannous fluoride; placebo (PLA)) and an experimental toothpaste with *S. baicalensis* extract (0.5%, plant extract (SB)) and stannous fluoride (1,400 ppm), too.

Randomization and supply of the products

All toothpastes were provided by Colgate-Palmolive, New York and supplied in identical tubes (labeled only with a code number of the toothpaste, the fluoride content, and expiry date) by a laboratory assistant who was not involved in the study. They were randomly allocated according to a random allocation table and determined by the order of attendance of the subjects for the initial treatment phase. The code was kept in a sealed envelope and was disclosed when all examinations were finished.

Study procedure

After an initial screening to ascertain the suitability of the subject to take part in the study and the completion of the Informed Consent, the volunteers were given a subject number by the order of attendance. At the beginning of the study, all subjects received a thorough oral prophylaxis (tartar removal and polishing with a rubber cup and the placebo toothpaste). Furthermore, an impression was taken from the lower jaw to build up the individual splint over the four teeth, which should develop gingivitis. Volunteers were instructed to maintain excellent oral hygiene for 10 days (pre-experimental phase) by brushing their teeth for a minimum of 2 min twice daily. The aim of this phase was to assure the least possible presence of plaque, as well as the practical non-existence of gingivitis, before the start of the experimental phase. For homogeneity, all subjects received a toothbrush and a toothpaste (the placebo dentifrice) for this hygiene phase.

After these 10 days, the subjects were called in to enter the study phase of the experimental gingivitis (baseline visit). The subjects were informed that from now on, they had to discontinue all oral hygiene procedures on four teeth

of the lower right quadrant (teeth 44–47) for a period of 21 days by using an individual plastic splint/shield to generate a possible gingivitis under the shield while they could perform their normal oral hygiene on the other teeth. After toothbrushing with the allocated toothpaste for 2 min, the subjects had to spit out the developed toothpaste slurry into a measuring cup. Then, they removed the shield and treated the (unbrushed) teeth (and the gingiva) with the toothpaste slurry, which means rinsing for 1 min. This had to be performed in the morning (after breakfast) and in the evening (after dinner). They also had to avoid rinsing afterwards with water, as well as eating or drinking within 30 min after using the slurry. After 14 and 21 days, the subjects were called in again for further assessment.

At each appointment, all the subjects were asked whether they have noticed any discomfort or new symptom. The use of systemic antibiotics or other antibacterial medications had to be reported and resulted in exclusion from the study. The occurrence of adverse events and side effects was also recorded at each visit.

Clinical evaluation

Gingivitis and supragingival plaque levels were assessed at days 0, 14, and 21 by means of the Löe and Silness Gingival Index [17] (primary parameter) and the Silness and Löe Plaque Index [18], respectively. The same researcher recorded all the measurements. The assessments were carried out on the mesial, distal, vestibular, and lingual faces of the four teeth which were protected by the shield during brushing.

Microbiological evaluation [biofilm vitality (VF)]

At days 0, 14, and 21, a plaque sample was taken from the teeth with a straight probe (EXS 9; Hu-Friedy), streaked on a microscope slide, and further processed in the laboratory using the vital fluorescence technique as described in Arweiler et al. (2006) [19]. After completing the staining reaction (with fluorescein diacetate and ethidium bromide), a cover glass was pressed firmly down onto the sample and the evaluation performed under a microscope (Axioskop 2 plus; Carl Zeiss, Göttingen). Using a digital camera (AxioCam HRc; Carl Zeiss, Göttingen), four pictures of different parts of each sample were stored. The vitality of the plaque sample (VF in %) was determined using an image analysis program to discriminate green (=vital) bacteria and red (=dead) bacteria (KS 300; Carl Zeiss, Göttingen, Germany).

On the last day of the study, each test subject received professional tooth cleaning and fluoridation of all teeth. The total duration of the study was 10 days for the hygiene phase and 21 days for the experimental phase.

Statistical analysis

Data from baseline (day 0), 14, and 21 days for each treatment and subject for Plaque and Gingival Index as well biofilm vitality were analyzed using the software SPSS Statistics 12.01 (SPSS Inc. Headquaters, Chicago, IL, USA). Gingival Index (GI) was the primary parameter. A between-treatment analysis (comparison between the two toothpastes) was conducted utilizing ANOVA. A within-treatment analysis (changes from baseline) was conducted using a paired t test at both 14 and 21 days. For all analyses, a difference was considered significant at the 95% confidence level (α=0.05). According to Shearer et al. [20] with a similar study design after 21 days we expected a mean GI of 1.4±0.07 with PLA and about 1.0±0.07 with an active paste. This corresponds to a 28% reduction, having 20 subjects per group. For the given input values, a power of >90% was calculated. A post-study calculation indicated that this study had a power of more than 90%.

Results

All 40 patients finished the study, 20 in the test group and 20 in the control group. No adverse or side effects during the study or later were reported. Means and standard deviations of all parameters assessed at the different time points are shown in Table 1.

Gingival index

The Gingival index (GI) in both the control (PLA) and the test group (SB) increased from baseline subsequently to the 14-day and 21-day follow-up. Besides baseline, data in the

Table 1 Mean (±SD) of GI, PI, and VF

	PLA (n=20)	SB (n=20)	p value
GI			
Baseline	0.038±0.058	0.028±0.050	0.563 (NS)
After 14 days	0.820±0.342	0.355± 0.243	<0.001; ***
After 21 days	1.585±0.218	0.934±0.239	<0.001; ***
PI			
Baseline	0.390±0.238	0.320±0.254	0.375 (NS)
After 14 days	1.835±0.383	1.270±0.285	<0.001; ***
After 21 days	2.250±0.383	1.700±0.229	<0.001; ***
VF			
Baseline	70.92±7.86	71.02±7.33	0.967 (NS)
After 14 days	83.08±5.18	79.80±6.99	0.100 (NS)
After 21 days	83.06±6.55	73.14±9.39	<0.001; ***

Statistical comparison of placebo versus test paste by ANOVA
NS non-significant; *** p<0.001

SB group were significantly lower than the control data ($p<0.001$) corresponding to a 41% reduction at day 21.

Plaque index

Plaque index (PI) raised continuously in both groups and revealed significant differences after 14 and 21 days ($p<0.001$).

Biofilm vitality

Biofilm vitality (VF) showed similar baseline values in both groups. SB revealed lower values after 14 and 21 days, which were only significant after 21 days.

Discussion

The study used a 21-day experimental gingivitis model. Gingivitis was experimentally induced in the study subjects by preventing cleaning of four teeth of the mandibulary right quadrant of the mouth using an individually adapted dental guard for each study participant.

This study model represents a further step in evaluating the antibacterial and clinical effect of oral hygiene products. Compared to 4-day plaque regrowth studies [19, 21–23], the experimental gingivitis design has a longer observation period to measure and evaluate gingival conditions (e.g., gingival index, bleeding on probing, and gingival crevicular fluid) concomitant with absence of mechanical hygiene and therefore without tempering with individual factors (different oral hygiene and better motivation: Hawthorne effect). This absence of mechanical hygiene is achieved by using the toothpaste as a slurry so that only the ingredients are tested. Ethical problems or problems with finding subjects are the disadvantages of this design. The modification with individual shields is often used to avoid an experimental gingivitis in the total mouth [7, 20, 24] and to better find subjects with a good compliance.

Generally, the findings of this study demonstrated significant advantages of the herbal toothpaste over the conventional fluoride paste at reducing plaque, gingivitis, and biofilm vitality after 21 days.

In the present study, all parameters increased continuously during the observation period of 21 days, GI increased under PLA to a value of 1.6, indicating a moderate gingivitis. At the end of the study, significant differences between both toothpastes could be seen, gingivitis was 41% reduced compared to the control paste. Obviously, the experimental toothpaste with the *S. baicalensis* extract exerted both an "anti-gingivitis" and "anti-plaque" effect. Both properties may have two causes: the less extent of gingivitis can be due to a direct anti-inflammatory effect on gingival tissue and also by less plaque formation due to an antibacterial effect. Conversely, as demonstrated previously [25, 26], the less extent of plaque formation can be due to less inflamed sites or—concerning the placebo paste—that the (worse) inflammatory status of the marginal gingiva had an important effect on plaque accumulation and significantly increased the amount of the novo plaque. However, the moderate (but significant) reductions in bacterial vitality concomitant with a pronounced gingivitis-reducing effect indicate a preponderance of anti-inflammatory properties of the test paste.

The findings of a similar experimental gingivitis study, where an anti-inflammatory drug (ibuprofen) was delivered systemically, demonstrated reduced gingivitis, but the drug failed to influence either the amount of plaque or the distribution of various bacterial morphotypes in the biofilm [6]. In contrast to that, a 1.5% dexiprofen mouth rinse had no effect on gingivitis whereas an anti-plaque effect was seen [7]. Heasman et al. [27] however were able to demonstrate superiority of a topical flurbiprofen rinse, whereas systemic absorption and effects cannot be excluded.

In general, the use of anti-inflammatory drugs is justified by the reduction of gingival fluid flow and hence, by influencing the build-up of plaque. However, it must be realized that—without antibacterial action—there seems to be no long-term effect on plaque by the suppressed gingival inflammation. In this context, the outcome of antimicrobial measures, mechanical as well as chemical, in the prevention and treatment of gingivitis is more predictable than that obtained by the (systemic) administration of anti-inflammatory compounds [6].

S. baicalensis extract—however—seems to be a promising agent with antibacterial and anti-inflammatory properties, which have inspired new uses for modern disease categories, such as bronchitis, urinary tract infections, hepatitis, and inflammation of the oral cavity. Moreover, latest findings showing benefits of *S. baicalensis* in prevention or adjuvant therapy of cancer [13–16] should be extended to oral diseases and could give the topical use of this herbal extract a new dimension.

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