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Clinical and antibacterial effect of tea tree oil – a pilot study

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Abstract The aim of this clinical pilot study was to compare the effect of tea tree oil with the effect of water and chlorhexidine on supragingival plaque formation and vitality. Eight subjects were asked to refrain from any kind of mechanical oral hygiene for 4 days after professional tooth cleaning (day 0), and to rinse with water instead for 1 week, with chlorhexidine in a second and tea tree oil in a third test week. The plaque index (PI), which was evaluated daily (days 1–4), served as a clinical control parameter. On the last day of the study (day 4), the plaque covering the front teeth was stained, photographed, and therefrom the plaque area (PA; %) was estimated using a digital measuring system. Each day of the study (days 1–4), the sampled plaque was examined using a vital fluorescence technique. Tea tree oil reduced neither the clinical parameters (PI and PA) nor the vitality of the plaque flora significantly. Within the limitations of the study design, it was determined that a solution with tea tree oil – utilized as ordinary mouthwash – has no positive effect on the quantity or quality of supragingival plaque.

Key words Tea tree oil · Dental plaque · Oral bacteria · Mouthrinse · Vitality

Introduction

The essential oil of the *Melaleuca alternifolia*, known also as tea tree oil (TTO), has been used in medicine for almost 70 years [6]. TTO is a complex mixture of hydrocarbons and terpenes, consisting of approximately 100 components. The concentration of each component can vary strongly in different preparations, which may also influence their antibacterial activity [6]. The antibacterial characteristic of the TTO in humans is based solely on empirical information, while its efficacy against *Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus* spp., and *Candida albicans* has been proven in vitro [6, 7, 8, 10]. TTO showed antibacterial effects in vitro also against anaerobic bacteria found in the oral cavity, including for example *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans* [22]. This positive antibacterial action of TTO having been established in vitro against yeasts as well as obligate anaerobic and facultative anaerobic bacteria was transferred to benefits for oral hygiene without scientific evidence.

Today, TTO is a component of numerous products for oral hygiene, including toothpastes and mouthrinse solutions, and is said to be an effective agent against dental plaque. Therefore, the purpose of the present study was to test the antibacterial efficacy of a TTO solution on plaque growth in vivo in a clinical short-term trial using established test parameters.

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Materials and methods

Study population

After receiving the study information and informed consent, eight dental students and staff of the University of the Saarland, aged from 23 years to 34 years, volunteered for the study. Criteria for exclusion were the use of antibiotics or other medicaments within the last half year that could have affected plaque growth, poor oral hygiene, less than 20 teeth available for evaluation, fixed or removable orthodontic appliances, or partial dentures.

Study design

At the beginning of every test week (day 0), all participants were given a professional tooth cleaning. For the following four test days, they had to refrain from any kind of mechanical oral hygiene measures. Instead, they rinsed twice daily for 2 min with 15 ml of the allocated mouthrinse solution. Every test week was followed by a 10-day washout phase in which normal toothbrushing with a standard toothpaste was performed. For the first test week, the rinsing solution used was water as negative control (placebo), for the second week it was 0.1% chlorhexidine (CHX, positive control; Chlorhexamed fluid®), and for the last week it was TTO (ten drops of maxim® pure Australian tea tree oil, Maxim GmbH and Co. KG, Cologne). Being water insoluble, the TTO had to be dispersed in one tablespoon of milk as an emulsifying agent by the participants themselves. This mixture was diluted with 100 ml warm water, resulting in a final concentration of about 0.34% or 3400 ppm of the TTO. From this solution, 15 ml was used as a rinse. The participants showed up for index evaluation in the department at the same time daily.

Clinical evaluation

The plaque index (PI) was assessed according to Silness and Löe [24] on days 1–4. On day 4, the plaque covering the anterior teeth and the canines was stained with erythrosin and photographed, and the plaque area (PA) was then evaluated by a blinded examiner using a special computer program ("fluoro"® medvis, Homburg) and calculated as the percentage of the entire tooth area.

The PI was assessed in all patients in just one quadrant for each day (day 1 in the 1st quadrant, day 2 in the 3rd quadrant, day 3 in the 2nd quadrant and day 4 in the 4th quadrant) and only on premolars and molars, in order not to disturb the plaque growth on other teeth and the evaluation of the parameters on the following days.

Microbiological assessment

From those teeth used for the assessment of the PI, supragingival plaque samples were taken simultaneously from the buccal surfaces with a straight explorer (EXS 9; Hu-Friedy). These plaque samples were placed on a glass slide and immediately brought to the laboratory for vital fluorescence staining according to Netuschil et al. [14–16]. After completion of the staining reaction, a cover glass was tightly pressed onto the sample and the evaluation with the microscope was started. The vitality of the sample was assessed by one blinded examiner with the use of a modified counting grid according to Brecx et al. [4] and its percentage calculated (VF%; percentage of vital bacteria in the entire sample).

Statistical evaluation

For each rinsing solution, the mean values of the clinical parameters (PI and PA) and the vitality of the supragingival plaque flora were calculated. PI and vitality were assessed on days 1–4; data of the PA were only available from one of the days (day 4). For the statistical evaluation, the computer program Statistical Package of Social Science/SPSS 7.5.2G was used. With the PI data, no statistical analysis was performed because the clinical examiner was not blinded.

Data series of the rinsing solutions (PA and plaque vitality) and their differences to the placebo solution were examined for normal distribution using the Kolmogorow-Smirnow test. Since the data were normally distributed, a parametric test could be applied. Significant differences between the single rinsing solutions and the placebo solution were detected using Student's paired *t*-tests. No Bonferroni adjustments were made [19].

A level of significance of α=0.05 and a power (1-β) of 0.90 were set. A 20% reduction in PA and plaque vitality with a 10% standard deviation was considered clinically relevant. For the given input values, a minimum sample size of n=6 was computed by the software program "statistics" (updated on 6 November 1999) of the UCLA homepage (http://www.stat.ucla.edu/calculators/powercalc/) for two-sided null hypothesis H_0.

Results

All eight subjects participated in the whole study period. However, all of them complained about the intensive and unpleasant taste of the TTO solution, yet the compliance of the subjects was not affected. The latter was proven – as well as it could be – by interviewing the volunteers. The mean results are shown in Fig. 1, Fig. 2, and Fig. 3, and the statements of significance in Table 1 and Table 2. TTO reduced neither the PI on any of the days 1–4 nor the percentage of covered PA% on day 4 relative to the placebo solution (P>0.05). Also, no significant efficacy of the TTO solution was detected on the relative amount

Table 1 Mean (±SD) values of the plaque index (PI) and the vitality of plaque (VF%) on days 1–3, and significant differences in comparison with the placebo solution (*ns* not significant; *P≤0.05, **P≤0.01, ***P≤0.001). No statistical analysis was performed with the PI data. *TTO* tea tree oil; *CHX* chlorhexidine

	PI±SD	VF±SD	*P*
Day 1			
Placebo	0.5±0.2	83.5±6.8	
TTO	0.7±0.2	71.6±13.4	ns
CHX	0.6±0.1	58.6±12.5	**
Day 2			
Placebo	0.8±0.2	79.4±6.5	
TTO	0.9±0.1	75.8±12.7	ns
CHX	0.7±0.1	70.4±16.1	**
Day 3			
Placebo	1.2±0.2	84.7±9.6	
TTO	1.3±0.2	86.2±4.6	ns
CHX	0.8±0.2	71.4±9.4	**

Table 2 Mean (±SD) values of the plaque index (PI), the plaque area as a percentage (PA%) and the vitality of plaque (VF%) on day 4, and significant differences in comparison with the placebo solution (*ns* not significant; **P*≤0.05, ***P*≤0.01, ****P*≤0.001). *TTO* tea tree oil; *CHX* chlorhexidine

Day 4	PI±SD	PA±SD	*P*	VF±SD	*P*
Placebo	1.5±0.3	38.9±9.7		93.4±5.0	
TTO	1.5±0.2	35.2±10.7	ns	84.4±10.8	ns
CHX	0.9±0.1	12.3±8.3	***	69.3±18.2	**

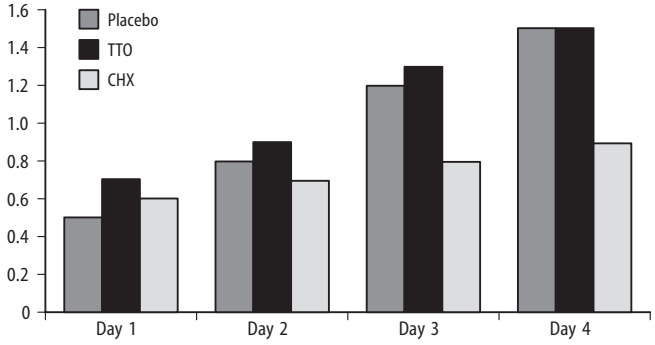

Fig. 1 Plaque index of each single rinsing solution during the course of the study

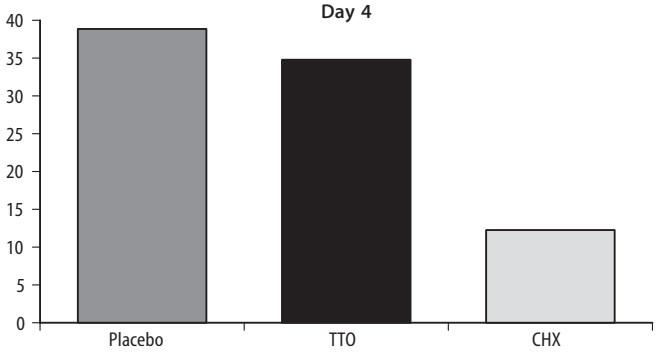

Fig. 2 Plaque area (%) of each single rinsing solution on day 4

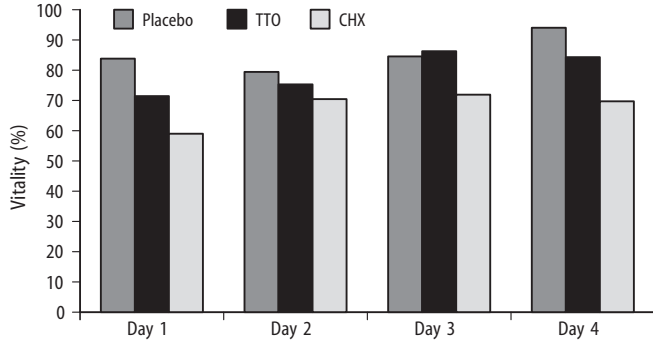

Fig. 3 Vitality of plaque (%) of each single rinsing solution during the course of the study

of vital bacteria (VF%) in supragingival plaque. Only a slight, insignificant decrease of VF% was found with TTO at days 1, 2, and 4.

CHX strongly reduced the PI only on day 3 and day 4. The percentage of PA was significantly reduced compared with placebo ($P<0.001$). CHX also had a significant effect ($P<0.01$) on the vitality of supragingival plaque on all of the four test days.

Discussion

The present short-term study was occupied with the clinical and antibacterial efficacy of a TTO solution on the supragingival plaque growth and the vitality of the plaque micro-organisms. For comparison, water (negative control) and CHX (positive control) were used. The classical design used for positioning the effect of a new antimicrobial agent is often applied by many authors in a similar way [1–3, 11, 12, 18]. All studies examined the short-time plaque regrowth; the tested product being the only hygiene measure for the following 4 days after professional tooth cleaning, which represents the baseline values. Moran et al. [12] pointed out that an antimicrobial product proving itself ineffective during this kind of short time study is not likely to have any effect when used as supplementary product for mechanical oral hygiene. The present study does not conform with the classic design in the way that studies mentioned above are cross-over, Latin-square randomized or double blind. In the present study, only the examination of the plaque index was not blinded, but the assessment of the vitality and the plaque area was made by a blinded examiner. Therefore, the plaque index was an additional index and was not evaluated statistically. Being water insoluble, the TTO solution had to be mixed by the participants themselves and, therefore, it was impossible to blind the volunteers.

The classic Latin square design whereby each treatment follows the other treatments the same number of times was not essential in this pilot study, because a relatively long washout phase of 10 days followed each study period contrasting some other studies which used only 2.5 days [3, 12, 13]. Newcombe et al. [18] recommended a washout period of preferably 10 days, because CHX still has a detectable effect after 3 days washout. The fact that TTO, which was used after the CHX, did not show any significant inhibiting effect relative to the negative control indicates that the washout period was sufficient to avoid carry-over effects of the CHX.

By the PI, a statement could be made about the amount of plaque forming under the influence of a mouthrinse solution. The incline of the PI during the four consecutive days as well as the general value of the PI values was in accordance with published findings [1, 2, 11, 16, 18], as was the retardation of the plaque accumulation with CHX [1, 2, 4, 11, 16, 18], although in these studies other PI values were used. Plaque accumulation on the teeth surfaces could be assessed even more precisely by knowing the percentage of

the area covered by plaque, because with it a continuous and more exact measurement of the amount of plaque is possible. Quirynen and van Steenberghe [20] considered the different PI values to not be exact enough and used also the calculation of the relative PA for a more detailed analysis of plaque growth for a period of 96 h. For evaluation, a planimeter was used by these authors. The amount of plaque was given as a percentage of the total buccal surface just like performed in the present study. Similarly, Addy et al. [1, 2] used a modification of the extrinsic stain index in their studies according to Shaw and Murray [23], in which the labial tooth surfaces of the eight anterior teeth were separated into 400 quadrates. The plaque-covered quadrates were then registered using a planimeter as well. In the present study, a computer program was used to calculate the percentage of the plaque-covered teeth surfaces similar to Söder et al. [25] and Eaton et al. [9]. Despite the fact that our data cannot be compared directly with other studies, the relationship between PA in the negative control (water, 39%) and the positive control (CHX, 12%) is in line with these recent findings.

For the assessment of antibacterial efficacy, not only the quantity of the plaque is of importance, but also its quality. Thereby, the vital fluorescence technique offers the opportunity to differentiate between dead and vital bacteria, thereby demonstrating the retardation of plaque formation by a mouthrinse [4, 5, 16, 21].

Concerning the obvious difference between the antibacterial action of TTO in vitro [6, 7, 8, 10] and its meager efficacy against the plaque biofilm in vivo, it should be kept in mind (1) that the MIC (minimum inhibitory concentration) data did not give direct information about a bactericidal effect, and (2) that the concentrations necessary to affect a bacterial biofilm are much higher (e.g., 100×) than those necessary to kill planctonic bacteria [17].

Regarding TTO, the established results cannot be compared with other in vivo studies, because there are no studies dealing with the antibacterial efficacy of TTO on dental plaque in the literature. By comparison with a positive and negative control, however, it was possible to classify the effect of TTO. The results show that TTO – used as simple mouthrinse solution – cannot effectively influence the quantity and quality of supragingival plaque.

Conclusion

Within the limits of the present study design, there is no effect of TTO on plaque regrowth and on the vitality of supragingival microflora.

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