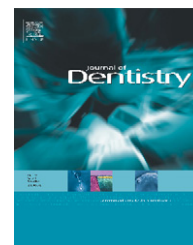


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Oral malodor reduction by a palatal mucoadhesive tablet containing herbal formulation

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ABSTRACT

Objective: The aim of the present study was to test the effect of a palatal mucoadhesive tablet containing an herbal formulation on oral malodor production and volatile sulfide compound (VSC) levels, and to evaluate its antimicrobial activity.

Methods: A total of 56 healthy young volunteers participated in experiments 1 and 2. The palatal adhesive tablets were prepared with different active ingredients (herbal formulation, zinc and chlorhexidine), or without an active ingredient as control (placebo). Measurement included odor judge scores (two judges) and VSC readings by a sulfide monitor (Halimeter[®]). In experiment 3, the antimicrobial activity of the herbal formulation ingredients (i.e. sage, Echinacea, Lavender and Mastic gum) were tested against three oral pathogens (*Streptococcus mutans*, *Porphyromonas gingivalis* and *Candida albicans*) by the agar diffusion test.

Results: Application of the palatal adhesive tablets containing herbal formulation resulted in a significant reduction in both oral malodor scores ($p < 0.001$) and VSC levels ($p = 0.013$). Herbal formulation showed higher significance in VSC reduction ($p = 0.001$), as compared to zinc and chlorhexidine ($p = 0.024$ and 0.032 , respectively). Sage, Lavender and Mastic gum showed antimicrobial activity against all three oral pathogens.

Conclusions: Results of the present study suggest that the palatal adhesive tablets containing herbal formulation may serve as an effective means of treatment for patients complaining of oral malodor.

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1. Introduction

Oral malodor is a common complaint among dental patients. This condition derives in most cases from the proteolytic activity of anaerobic Gram-negative oral bacteria such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Prevotella intermedia*.^{1–3} These bacteria reside on various locations within the oral cavity (e.g. tongue dorsum, interdental space, period-

ontal pockets, faulty and leaky restorations and tonsils) and they breakdown salivary and oral proteins into their amino acid building blocks. Some of these amino acids (e.g. methionine and cysteine) are further metabolized yielding malodorous volatile sulfide compounds (VSCs) such as methylmercaptan and hydrogen sulfide.⁴

The tongue dorsum and especially its posterior portion are considered the key location for this process.⁵ Therefore, the

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treatment regimen includes in most cases a daily use of tongue scrapers and mouthwashes. Indeed, a recent Cochrane systematic review found tongue scrapers to have short-term efficacy in controlling halitosis.⁶ However, the use of tongue scrapers is unpleasant and induces in many cases a strong gag reflex, and apparently has very little effect on the bacterial load of the tongue.⁷ Moreover, some mouthwashes have been shown to cause adverse side effects such as tooth staining.⁸

The use of buccal adhesive tablets comprised of mucoadhesive polymers and their sustain release abilities for water-soluble medicinals have been previously reported.^{9,10} In the present study, we performed a set of related experiments on such a delivery system in the form of a palatal mucoadhesive tablet containing an herbal formulation. Locating the mucoadhesive tablet to the palate places it right above, and in direct contact with the tongue dorsum, thus allowing a sustained release of the active ingredients directly to the target site. The ingredients of the herbal formulation (i.e. sage, Echinacea, Lavender and Mastic gum) have been previously shown to demonstrate antimicrobial activity.^{11,12} Furthermore, these four natural medicinals were selected for their abilities to reduce malodor and VSC production and inhibit proteolysis as water-soluble materials in a salivary incubation assay.¹³ We tested the effect of this system on oral malodor production and VSC levels in a young healthy population, and the antimicrobial effect of its active ingredients on three known oral pathogens (*Streptococcus mutans*, *P. gingivalis* and *Candida albicans*).

2. Materials and methods

2.1. Preparation of adhesive tablets

The adhesive polymers hydroxypropyl cellulose (Hercules Co., Wilmington, DE), and carbopole (Goodrich Co., Cleveland, OH) were mixed in a ratio of 4:1. 250 mg of the mixture with or without an active ingredient (as control) were pressed for 30 s at a pressure of 3 ton/cm² into a mold using a laboratory Carver press (Carver Machine Works, Inc., IN, USA). This process produced tablets of 12 mm in diameter and 2.5 mm in thickness with one side flat and the other side curved to fit the shape of the palate.

The active ingredient was an herbal formulation composed of equal shares of four herbal medicinals: Echinacea (*Echinacea augustifolia*), Mastic gum (*Pestacia lentiscus*), Lavender (*Lavandula augustifolia*) and Sage (*Salvia officinalis*), supplied as dried powders (Herbalife Co. Lod IL).

2.2. Study population

A total of 56 healthy young volunteers participated in the study: (i) 26 volunteers participated in experiment 1 (mean age, 29.3 ± 9.9 years, 10 females), and (ii) 30 volunteers participated in experiment 2 (mean age, 27.3 ± 5.2 years, 14 females). Volunteers who were smokers or took antibiotics within 1 month prior to the study were excluded. Informed consent was obtained and the experimental protocol was

approved by Helsinki committee and registered at the NIH-FDA protocol registration system (NCT-000250289).

2.3. Experimental protocol

Volunteers were asked to refrain from any kind of oral activity such as: eating, drinking, brushing, oral rinsing or gum chewing for 2 h prior to measurements. They were also instructed not to use commercial mouth rinses or other breath fresheners on the day of the experiment. Measurements (described in detail below) included odor judges' scores and volatile sulfide measurements.

2.3.1. Experiment 1—oral malodor reduction

Volunteers were randomly assigned into either of two treatment groups: (i) palatal adhesive tablet containing herbal active ingredients (treatment group, $n = 15$) and (ii) palatal adhesive tablet without any active ingredients (placebo, $n = 11$). Following baseline measurements of oral malodor-related parameters by two odor judges and a sulfide monitor, volunteers were instructed to apply the adhesive tablets to their palates. Measurements were repeated twice more (60 and 120 min) following application.

2.3.2. Experiment 2—VSC reduction

Volunteers were randomly assigned into one of four treatment groups: (i) palatal adhesive tablet containing zinc gluconate (10 mg) as an active ingredient (zinc group, $n = 7$), (ii) palatal adhesive tablet containing herbal formulation (10 mg) as an active ingredient (herbal group, $n = 8$), (iii) palatal adhesive tablet containing chlorhexidine gluconate (0.12% w/w) as an active ingredients (chlorhexidine group, $n = 7$) and (iv) palatal adhesive tablet without any active ingredients (placebo group, $n = 8$). Following baseline measurements of intra-oral VSC levels using the sulfide monitor, volunteers were instructed to apply the adhesive tablets to their palates. Measurements were repeated twice more (60 and 120 min) following application.

2.3.3. Experiment 3—antimicrobial activity

The antimicrobial activities of the four herbal medicinals against three known oral pathogens (*S. mutans* ATCC 27351, *P. gingivalis* ATCC 33277 and *C. albicans* CBS 562) were evaluated using an agar diffusion test. This test was performed by impregnating 6 mm paper discs with the ethanolic extracts of each herb as previously described.¹⁴ The extracts were prepared at varying concentrations (0.5, 1, 2, and 4% w/v) and the impregnated discs were dried and applied on various agar plates sectored and plated with the different microorganisms (100 μ L, 1 OD): (i) brain heart infusion (BHI) agar supplemented with bacitracin and glucose for *S. mutans*, (ii) "Willkin's agar" selective-enrichment agar for *P. gingivalis*, and (iii) yeast peptone dextrose (YPD) agar for *C. albicans*. Additional paper discs were impregnated with ethanol alone and served as negative control or with Listerine[®] (Cool Mint[®], Warner-Lambert, Inc., NJ) as positive control. Plates were incubated at 37 °C under anaerobic (for *S. mutans* and *P. gingivalis*) or aerobic (for *C. albicans*) conditions for 72 h. Following incubation the bacterial growth inhibition zone diameters were measured. The experiment was done in six replicates.

2.4. Measurements

2.4.1. Organoleptic measurements

Oral malodor was scored by two trained and calibrated odor judges, who were blinded to one another's scores, as well as to the other data. Volunteers were instructed to exhale briefly through the mouth, at a distance of approximately 10 cm from the nose of the judge. Malodor was scored using a semi-integer scale of 0-5 with description as follows: 0, no odor; 1, barely noticeable odor; 2, slight but clearly noticeable odor; 3, moderate odor; 4, strong odor; 5, extremely strong odor.¹⁵

2.4.2. Sulfide monitor

Intra-oral headspace volatile sulfide levels were determined by means of a portable sulfide monitor (Halimeter[®], Interscan) as previously described.¹⁶ Volunteers were asked to refrain from talking for 5 min prior to measurements. The monitor was zeroed on ambient air, and the measurements were performed by the insertion of a disposable 1/4-in. plastic straw approximately 5 cm into the partially opened oral cavity. Volunteers were asked to breathe through their noses during measurements. Results were recorded as peak ppb hydrogen sulfide equivalents.

2.5. Statistical analysis

To compare the quantitative variables (VSC levels and inhibition zone diameters), we applied ANOVA with *post hoc* pairwise comparisons, according to Dunnett and Scheffe. For the rank variables (odor judge scores), the Mann-Whitney non-parametric test was applied for pairwise comparisons, with the Bonferroni correction utilized to determine significance levels. All the tests applied were two-tailed, and $p \leq 0.05$ was considered statistically significant.

3. Results

Results of experiment 1 are presented in Fig. 1a and b. The treatment group showed significant reduction of 67% in malodor ratings ($p < 0.001$), and 64% in VSC levels ($p < 0.001$), following treatment as compared to the control. The treatment group maintained malodor ratings of under 1.5 and VSC levels of under 100 ppb during the whole experiment. No change was observed in both malodor-related parameters in the placebo group, and malodor and VSC levels remained high (over 3 and 160 ppb, respectively). No difference was observed between the two groups at baseline.

Results of experiment 2 are presented in Fig. 2. All the active ingredients showed significant reductions in VSC levels. However, the herbal formulation showed a highly significant reduction ($p = 0.001$) as compared with the moderate significance of zinc and chlorhexidine ($p = 0.024$ and 0.032 , respectively). The herbal active ingredient did cause the highest VSC level reduction as compared to the other active ingredient, however these differences were not statistically significant.

Results of experiment 3 are presented in Fig. 3a-c. As demonstrated by the results of the agar diffusion test, both Sage and Lavender showed significant antimicrobial activity

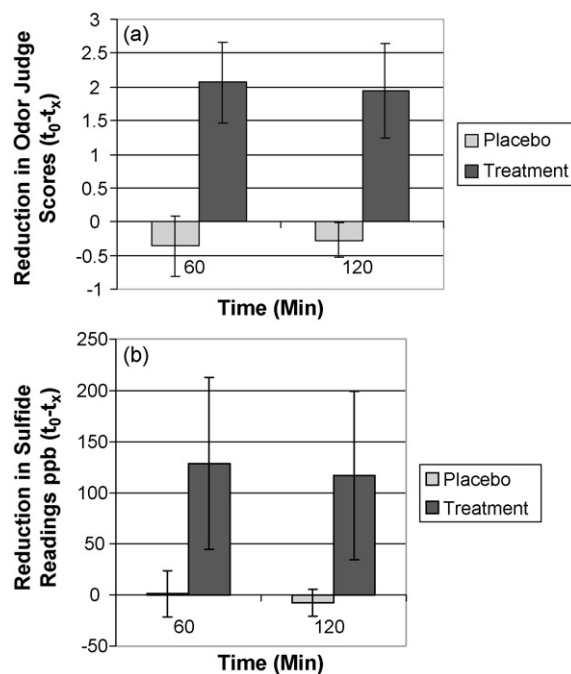


Fig. 1 – Effect of palatal adhesive tablets on: (a) odor judge scores reduction compared to time 0, as recorded on a 0-5 scale, and (b) reduction in volatile sulfide compounds (VSCs) compared to time 0, measured as ppb sulfide equivalents using a sulfide monitor (mean results and standard deviation).

against *S. mutans* as compared with the negative control ($p < 0.001$ for the 4% Sage extract, and $p = 0.021-0.001$ for 1-4% of Lavender extract). Moreover, the activity of the Sage extract (4%) against *S. mutans* was significantly higher than the positive control ($p = 0.027$). Only Lavender extract (4%, $p < 0.001$) and Mastic gum extract (0.5-4%, $p < 0.001$) showed antimicrobial activity against *P. gingivalis* (Fig. 3b). None of the other materials, including the positive and negative controls showed any activity against this bacterium. All of the extracts

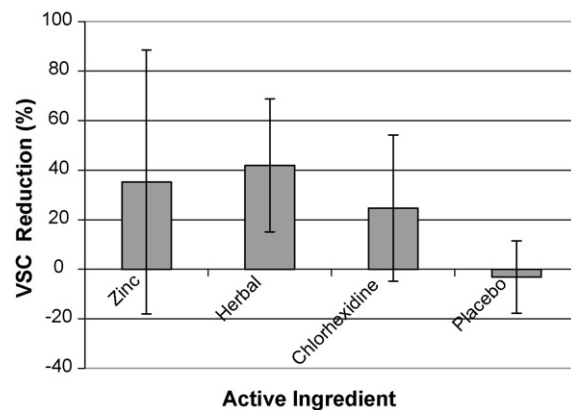


Fig. 2 – Effect of various active ingredients, incorporated into the palatal adhesive tablets, on percent reduction of volatile sulfide compounds (VSC) (mean results and standard deviation).

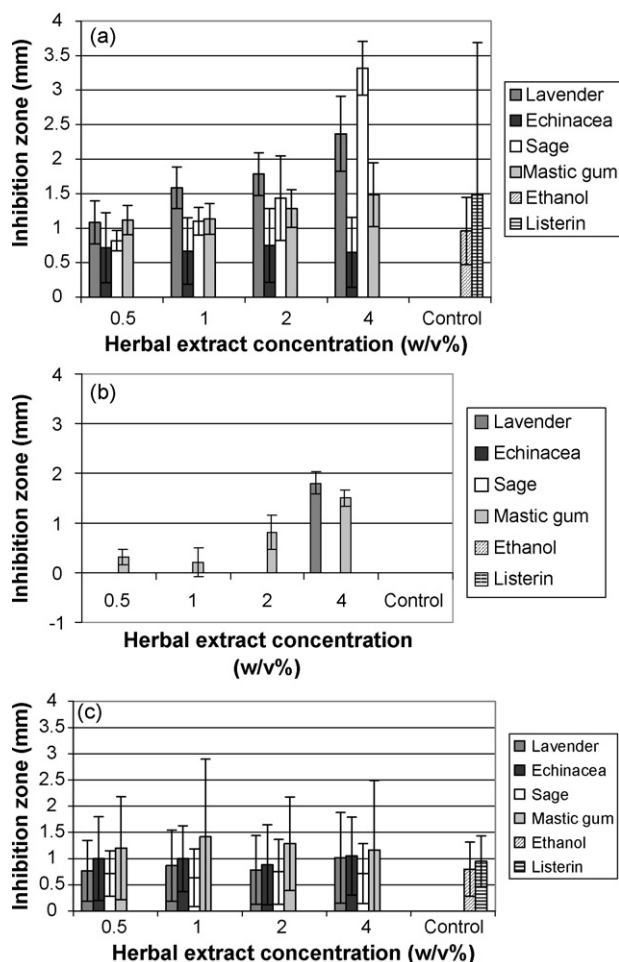


Fig. 3 – Antimicrobial effects of the various ingredients of the herbal formulation against the tested oral pathogens: (a) *Streptococcus mutans*, (b) *Porphyromonas gingivalis*, and (c) *Candida albicans*, as measured by the agar diffusion test (mean results and standard deviation).

and the controls showed antimicrobial activity against *C. albicans* (Fig. 3c). The highest activity was demonstrated by the Mastic gum extract. However, these differences were not statistically significant.

4. Discussion

Previous study demonstrated the strong antimalodorous activity of Lavender, Echinacea, Sage and Mastic gum in a salivary incubation assay.¹³ These herbs demonstrated antimicrobial activities, VSC conversion properties and proteolysis inhibition abilities. This suggested, that these natural medicinals might serve as effective agents in oral malodor treatment. In the present study, we incorporated the herbal formulation comprised of these four herbal medicinals into a slow dissolving mucoadhesive tablet, designed to fit the palate.

Results indicated that the adhesive tablet containing the herbal formulation is effective in reducing oral malodor and VSC levels. Experiment 1 showed that malodor levels were

reduced below noticeable levels (odor judge scores < 1.5), following treatment with the active tablets, and stayed low for the entire duration of the experiment. The fact that malodor levels in the placebo group stayed high (odor judge scores > 3) confirm that the herbal formulation is indeed the active cause for this reduction.

In experiment 2, the herbal formulation showed higher significance in VSC reduction as compared to the other active ingredients tested (*i.e.* zinc¹⁷ and chlorhexidine). These ingredients are commonly used as antimicrobial agents in various dentifrices. Despite their common use both of these active ingredients have their drawbacks. For instance, chlorhexidine has been reported to cause side effects such as tooth staining,⁸ and zinc has an unpleasant taste. Therefore, the search for better and friendlier active ingredients is certainly a valid one.

Experiment 3 demonstrated the antimicrobial effect of the herbal ingredients against the three oral pathogens implicated in causing various diseases in the oral cavity such as: caries, periodontal disease and oral thrush. The antimicrobial activity of the various herbal medicinals, especially Sage, Lavender and Mastic gum, suggests that other oral conditions may benefit from this treatment.

This delivery system presents two distinct advantages: adhesiveness and sustained release. The adhesiveness of the tablet enables it to stay in place, thus maintaining constant contact with the target site. Furthermore, it allows the patient to carry on with his regular activities (*e.g.* eating, drinking and talking) without interference, as compared to breath mints and lozenges. The slow dissolving of the tablet enables a prolonged exposure of the bacteria to the active ingredients and improving their effectiveness.

Results of the present study suggest that the palatal adhesive tablets containing herbal formulation may serve as an effective treatment for patients complaining of oral malodor. Further research, establishing the duration of effectiveness, recommended dosages and dissolving time is currently on the way.

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