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ORIGINAL ARTICLE

The effect of bee propolis on recurrent aphthous stomatitis: a pilot study

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Abstract Recurrent aphthous stomatitis (RAS) is a common, painful, and ulcerative disorder of the oral cavity of unknown etiology. No cure exists and medications aim to reduce pain associated with ulcers through topical applications or reduce outbreak frequency with systemic medications, many having serious side effects. The purpose of this pilot study was to evaluate the potential of a product to reduce the number of outbreaks of RAS ulcers. Propolis is a bee product used in some cultures as treatment for mouth ulcers. In this randomized, double-blind, placebo-controlled study, patients were assigned to take 500 mg of propolis or a placebo capsule daily. Subjects reported a baseline ulcer frequency and were contacted biweekly to record recurrences. Data were analyzed to determine if subjects had a decrease of 50% in outbreak frequency. The data indicated a statistically significant reduction of outbreaks in the propolis group (Fisher's exact test, one sided, $p=0.04$). Patients in the propolis group also self-reported a significant improvement in their quality of life ($p=0.03$). This study has shown propolis to be effective in decreasing the number of recurrences and improve the quality of life in patients who suffer from RAS. Propolis should be evaluated further in a larger sample clinical trial.

Keywords Propolis · Recurrent aphthous stomatitis · Aphthous ulcers · Clinical trial · Evaluation study

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Introduction

Recurrent aphthous stomatitis (RAS) is a common condition that affects approximately 20% of the general population [9, 17, 21, 22]. It is a painful inflammatory ulcerative disorder of the non-keratinized oral mucosa that can cause problems with eating, swallowing, and speaking. Typically, the clinical presentation consists of a prodrome of burning, itching, or localized pain preceding ulcer formation for 24–48 h. A distinct, shallow, round ulcer with a necrotic center covered by a pseudomembrane forms and is surrounded by a raised, erythematous halo around the border. Pain usually abates after 4 days with the beginning of re-epithelialization [9, 21]. Slightly more females suffer from RAS than males and there is an increased prevalence in high socioeconomic status groups. Eighty percent of cases of RAS have onset before the age of 30 [9, 17, 21, 22].

As no definitive etiology has been identified, treatment of RAS is challenging. Treatment is symptomatic and should be tailored to the pattern and severity of each patient. Goals that may be addressed are a decrease in symptoms, a reduction in ulcer number and size, and an increase in the disease-free periods [9, 21]. Few randomized controlled clinical trials have been conducted to determine treatment for RAS. Chlorhexidine has been shown to decrease severity and duration, but not the frequency of ulcer occurrence, leading investigators to point to an infectious agent as the cause of RAS [15, 21]. Similarly, topical steroids have also been shown to decrease severity and duration of ulcers, implicating deregulation of the immune system as the causative process [15, 21]. None of these medications have proven to be effective in all RAS patients. Given the severe side effects and limited efficacy of the current treatment, this study investigates a new treatment for the prevention of RAS ulcers.

Propolis is an over-the-counter, flavonoid-containing food supplement. In vitro, flavonoids such as those found in propolis have demonstrated antimicrobial activity, free-radical scavenging ability, immune system activation, and numerous antioxidant properties [1, 5, 6, 8, 12, 16, 19]. As such, propolis has been assessed in the treatment of a variety of inflammatory and ulcerative conditions [10, 11, 21] with low rates of minimal side effects, including contact dermatitis [7, 19]. The use of propolis for the treatment of mouth ulcers is a traditional therapy utilized by some communities in the Middle East. In a preliminary study by Samet et al. (unpublished data) patients who took a 500-mg supplement of propolis daily were shown to have a statistically significant decrease in the frequency of outbreaks of RAS. Additionally, multiple studies report a positive effect of flavonoids on gastrointestinal ulcers [2, 13, 14]. This knowledge, coupled with the product's availability and low risk of side effects, initiated this study to test the effects of propolis as a preventative agent for aphthous ulcers. The level of 50% reduction in frequency was used as a standard, with a goal of approximately 65% of patients reaching that level of reduction [4]. We hypothesized that daily ingestion of one 500-mg capsule of propolis will decrease the frequency of outbreaks of recurrent aphthous stomatitis in patients when compared with subjects in the placebo group.

Materials and methods

Study population The sample consisted of 19 patients suffering from RAS minor at a minimum frequency of four outbreaks per year. Study subjects were recruited from patients presenting for treatment at the Harvard School of Dental Medicine (HSDM) clinics, members of the local community, and word-of-mouth referrals. Patients were recruited over a 10-month period. Patients from the Boston area who were interested in enrolling in the trial presented to HSDM with an active aphthous ulcer to confirm diagnosis and enroll in the study. Patients not in the Boston area were asked to have a medical diagnosis of RAS minor before enrollment. Patients were at least 18 years old at the time of enrollment and did not have RAS associated with other conditions such as anemia, vitamin deficiencies, inflammatory bowel disease, celiac disease, Behcet's disease, Reiter's disease, or HIV-associated immunosuppression. Exclusion criteria also included a history of allergy to propolis, bee products, or bee sting. Patients were asked to participate in the study for a minimum of 6 months. The study was approved by the institutional review board for human studies (IRB #M10778-101).

Subjects were informed that, although there is no conventional scientific study to support propolis as a treatment, anecdotal evidence suggests it may play a role in the reduction of pain and frequency of RAS-associated ulcers. Subjects were also informed that possible allergic reactions may occur and that they should terminate use of the product if any adverse reactions are noted. Patients were also aware that they may have received either the propolis or a placebo and that they would be informed of their assignment at the termination of the study. All patients would be offered additional propolis upon completion of the trial. Patients were given written and oral explanation of the study and risks and benefits.

Patients were randomly divided into two subgroups: the propolis group ($n=10$), who received a daily dosage of 500 mg/day of bee propolis (Vitamin World), and the placebo group ($n=9$), who received a daily placebo capsule of a calcium-based food supplement. As this was a double-blind study, neither the participants nor the investigators knew the identity of the product distributed. The investigators received prepackaged bottles of pills, which were only identified by a colored label. The identity of the supplement was not revealed until after data analysis was completed. The patients were asked to swallow one capsule of the material they were given each day. They were asked not to use any other product for the prevention or treatment of aphthous ulcers while participating in this study. All participants were told that after data analysis, if a benefit was seen from usage of 500 mg/day of propolis, all patients would be offered an additional 3-month supply of propolis, regardless of their initial group status.

At the initial intake and diagnosis session, patients were asked to self-report their frequency of RAS outbreaks. Patients were also asked about other medications tried to prevent or alleviate pain from RAS and any current illnesses for which they are receiving medical care. A biweekly phone call or e-mail was conducted by one of the investigators to ensure compliance with product intake and to gather information regarding recurrence of aphthous ulcers. Patients were asked to report the frequency of taking the supplement, the number of new aphthous ulcers in the 2-week period, and the duration and subjective severity (on a 1–10) scale of the ulcers. Participants were followed for the duration of their enrollment in the study.

Statistical methods Descriptive statistics were performed to ensure that the two groups were comparable with regard to the length of participation in the study and self-reported number of annual aphthous ulcers. Based on the self-reported frequency of aphthous ulcers, the number of ulcers expected during the enrollment in the study was calculated. We compared those expected values with the actual number of ulcers reported by the patient during the trial to obtain a percent reduction in RAS outbreak frequency.

Fisher's exact test was used to analyze statistical significance between the number of patients with a specific level of

reduction in frequency between the two groups (p=0.050). Based on the study results from Samet et al. (unpublished data), we felt a one-sided test could be used to analyze the data, since this data indicated that propolis does not cause an increase in recurrence frequency but rather a decrease. However, for completeness, we also analyzed the data with two-sided tests. The number of patients enrolled in this study is small; however, analysis shows a statistical significance in the one-sided analysis and near significance for the two-sided tests. The level of reduction analyzed was ≥50% decrease in frequency in outbreaks. Fisher's exact test was used to compare and assess the number of patients in the two groups who reported an improved quality of life. All statistical analyses were conducted in SPSS (v11.0, © SPSS, Chicago, IL).

Results

Of the 19 subjects enrolled in the study, two patients (10.5%) did not complete at least 6 months of follow-up. One was unhappy that he was not seeing any results from the daily supplement and wished to terminate his participation at 3 months. A second patient noticed an outbreak of acne and, at the advice of his dermatologist, terminated participation at month 5. Both of the patients were in the placebo group. The other patients participated from 6 to 13 months. Although patients were asked to enroll in the study for a minimum of 6 months, all patients were included in the data analysis, as the data were adjusted for time enrolled. Overall, the compliance rate in both the placebo and propolis groups was approximately 90% and the noncompliance events were evenly distributed among all participants.

Descriptive statistics for the study groups are summarized in Table 1. There were no statistically significant differences between the groups with regard to follow-up time, reported annual number of outbreaks before treatment, and the expected number of outbreaks. Patients in the propolis group experienced a mean outbreak reduction of 26.5% compared to 12.5% in the placebo group. This difference was statistically significant at the 0.05-level using a one-sided test.

Table 1 Descriptive statistics for study groups

Variable	Propolis group (n=10)	Placebo group (n=9)	p Value
Follow-up time (years)	0.68±0.14 (0.50–0.92)	0.63±0.25 (0.21–1.1)	0.33
Previous reported annual sores	11.8±7.3 (4.0–24.0)	14.0±7.4 (4.0–24.0)	0.28
Expected number of sores	8.0±4.8 (2.0–16.0)	8.3±4.8 (2.0–16.0)	0.46
Observed number of sores	4.4±4.3 (0.0–14.0)	7.0±5.4 (2.0–19.0)	0.14
Percent reduction in sores	26.5±94.3 (−200.0 to 100.0)	12.5±35.1 (−52.0 to 75.0)	0.05

All p values listed are one-sided p values.

Analysis at the level >50% reduction in frequency of outbreaks Six patients (60%) in the propolis group and one patient (11%) in the placebo group experienced this level of reduction (Table 2). The null hypothesis is that there is no difference in the proportion of patients with ≥50% reduction between the placebo and treatment groups. The two-sided alternative hypothesis is that there is a difference in proportion of patients with ≥50% reduction between the placebo and treatment groups, while the one-sided alternative hypothesis states that the proportion of patients in the propolis group with ≥50% reduction is greater than that in the placebo group.

The data above demonstrate that approximately 11% of patients in the placebo group had at least a 50% reduction in ulcers, compared to 60% in the propolis group. Using a one-sided Fisher's exact test, this result is statistically significant at the 0.05-level (p=0.040). The result is near statistical significance for the two-sided test (p=0.057).

Analysis for improved quality of life Patients were not asked to comment on whether they believed their supplement to be having an effect on their quality of life or frequency of outbreaks. However, in the propolis group, five patients volunteered statements without prompts indicating their quality of life had improved. For example, one patient said that taking this drug has "changed [her] life" and another reported that she is able to eat her personal "trigger foods" for the first time in many years. Patients who offered this positive feedback often expressed thanks to the investigator when called on biweekly follow-ups for helping return their lives to normal. At the end of the study, several patients enthusiastically asked for more propolis so they could continue to take it on a regular basis. No patients in the placebo group offered similar sentiments. The only patients who dropped out of the study were in the placebo group, one stating that he was unhappy with the lack of results. These data are summarized in Table 3.

Table 2 Association between study group and 50% reduction

		Study group		Total
		Propolis	Placebo	
Was there >50% reduction?	Yes	6	1	7
	No	4	8	12
	Total	10	9	19

Fisher's exact test, one-sided p value=0.04 (two-sided p value=0.06)

Table 3 Association between study group and patient-reported improvement in quality of life

		Study group		Total
		Propolis	Placebo	
Was there improvement in QOL?	Yes	5	0	5
	No	5	9	14
	Total	10	9	19

Fisher's exact test, one-sided p value=0.03 (two-sided p value=0.02)

In this analysis, the null hypothesis is that there is no difference in proportion of patients reporting improved quality of life (QOL) between the two study groups. The two-sided alternative hypothesis is that there is a difference in proportion of patients reporting improved QOL between the two study groups. The one-sided alternative hypothesis is that the proportion of patients in the propolis group reporting improved QOL is greater than that in the placebo group.

Based on the data above, using Fisher's exact test, the result is statistically significant for a one-sided test (p=0.02) and for a two-sided test (p=0.03). Patients in the propolis group disproportionately reported improved quality of life, compared to their placebo counterparts.

Discussion

Recurrent aphthous stomatitis manifests in a variety of ways in patients who suffer from the disease. Currently, there is no known etiology for the ulcers nor is there a treatment that can safely and conclusively decrease the frequency of ulcer outbreaks in a patient. The present study shows that daily use of propolis may decrease the frequency of aphthous ulcers in patients. There is no gold standard for prevention of RAS and, therefore, no standard measurement against which to compare the reduction seen with a new potential treatment. Treatment of migraine headaches is similar in theory to treatment of RAS; patients could either treat symptoms or take medication for prevention. The review article on migraine treatments by Goadsby et al. [4] stated that acceptable medications for the prevention of migraines result in approximately 50% reduction in two-thirds of patients. We chose to use this level of reduction as our standard for evaluation of propolis.

At a 50% reduction in frequency, more patients in the propolis group reached the stated threshold.

Perhaps even more interesting in this study is the finding that taking 500 mg daily of propolis increases the quality of life for these patients. Aphthous ulcers can limit speaking, swallowing, and can cause a patient to avoid certain trigger foods or activities. Since the patients in this study volunteered this information, false negatives are a great concern. A standardized questionnaire regarding questions on QOL should be included in future studies. Five hundred milligrams was chosen as the study dosage from data in Samet et al.'s preliminary study and because this amount is readily available commercially. The dose of propolis should be evaluated in future studies.

One difficulty in performing a study such as this one is that the research is essentially "backward" from the normal design. Since the cause of RAS is unknown, one cannot begin with a pathology or etiology to treat and can therefore only speculate on the effects of propolis. However, given the broad biological composition of the substance, many theories are probable. If infectious agents are considered a cause of minor forms of RAS, then the antibacterial, antifungal, and antiviral activity of propolis may be the therapeutic mechanism. If the immune or inflammatory factors are etiologic, the various compounds such as flavonoids in propolis may target these pathways. At this time, however, such discussion is purely speculation. This study began with a treatment that was hypothesized to treat RAS and, hopefully in the future, can elucidate the mechanism of action. However, without pathological basis, IRB approval is difficult to obtain and requires an extensive review of existing literature before acceptance.

The greatest limitation of this study was the small sample size. Recruitment of patients occurred over a 10-month period by different advertising methods. Most patients that showed an initial interest, but failed to enroll in this study, wanted an abortive agent, not a preventive agent for their aphthous ulcers. When they learned they would be taking a preventive supplement each day, several potential candidates were no longer interested. More aggressive measures need to be taken to recruit more patients for future studies in this subject, including, possibly, financial compensation to participants. An additional difficulty was that, since this was a preliminary study, patients could not be assured that a benefit would be seen. Many patients were hesitant to enroll in a study with little scientific support, although they were told about the preliminary study and the suggestions of scientific data.

A larger sample size would allow for stronger statistical trends to be identified and also allow for grouping of patients to analyze more thoroughly in which patients propolis works most effectively. We propose using the same basic study format, however, with a sample of at least 100 subjects with varying degrees of severity of RAS. Patients who experienced fewer aphthous ulcers per year at baseline were in general less likely to take the pills on a daily basis and were less enthusiastic about the outcome of the trial. This is most clearly identified by the one patient who requested early termination from the study due to "no effect" from the product.

Another limitation in the study is the dependence on the self-reported nature of the baseline data. When questioned, many patients found it difficult to quantify the number of aphthous ulcer outbreaks they experienced each year. Many gave ranges for outbreaks, i.e., 10–12 outbreaks per year. If this was the information initially reported to investigators, in our statistical analysis, the baseline value use for this patient was 11 outbreaks per year. One study examining the effects of systemic medications for severe RAS minor evaluated patients for 1 month, treated patients for 2 months, then continued with two more months of follow-up [3]. This design was feasible because the patients all had high rates of recurrence. Monitoring patients for an extended period of time would be necessary due to the unpredictable cyclic nature of the disease. It is probable that patients both over- and underestimated the amount of annual RAS outbreaks, making the value of percent reduction an estimate. Ideally, one would observe the patients for 1 year before intervening with trial supplement, but that is very difficult in this clinical setting. Our study was designed to follow patients for at least 3 months, but ideally 6 months based on the inconsistent pattern of outbreaks in RAS sufferers. This design should allow for the evaluation of the effect of propolis, even considering the seemingly random outbreak pattern.

It appears as though propolis may confer some advantage in the treatment and management of patients with aphthous ulcers—the trend suggests that patients taking propolis are more likely to achieve reductions in number of aphthous ulcers, compared to those patients on placebo. A larger sample size may allow for more rigorous discrimination between the effects of propolis and placebo in this regard. It is important to note that self-reported improvements in quality of life were statistically significantly higher for patients in the propolis group vs those in the placebo group.

While a study with a larger sample size is necessary, this trial shows that daily ingestion of 500 mg of propolis may lead to a decrease in aphthous ulcer outbreaks and an improvement in a patient's quality of life. On the basis of the results in this study, we advocate the use of propolis for patients with RAS who do not respond to other forms of treatment. We hope that a larger scale study will allow for propolis to become a first-line therapy for all patients with RAS.

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