Effect of the Use of Propolis on Serum Levels of Interleukin-17 and Clinical Symptoms and Signs in Patients with Ulcerative Oral Lichen Planus

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ABSTRACT

Background and aims: Ulcerative oral lichen planus is a relatively common symptomatic condition with a potential for malignant transformation. Interleukin-17 is a cytokine, which has attracted the attention of researchers as a factor effective in its etiology. Therefore, recent research in relation to the treatment of inflammatory and immunologic diseases has focused on techniques to decrease and regulate cytokines, especially interleukin-17. Propolis is one of the products of honeybees and has anti-inflammatory, antineoplastic and antimicrobial properties and an effect on decreasing IL-17 levels. The aim of the present study was to evaluate the effect of propolis on serum levels of IL-17 and clinical symptoms and signs in patients with ulcerative oral lichen planus.

Materials and methods: In this clinical trial, 25 patients with ulcerative lichen planus were included. Blood samples were taken to determine IL-17 serum levels. Then 500 mg capsules of propolis were given for 30 days a capsule daily. After a month, serum levels of IL-17 were determined once again. In addition, pain severity (VAS) and the maximum sizes of lesions before and after administration of propolis were determined. Results: Mean serum levels of IL-17 before and after intervention were 135±16.5 and 127±12 pg/mL, respectively, demonstrating statistically significant differences. In addition, VAS means before and after treatment were 4.2±3.6 and 3.3±2.8, respectively, with statistically significant differences. The means of maximum lesion sizes before and after treatment were 14±7.7 and 9.8±7.6 mm, revealing statistically significant differences. Conclusion: Administration of propolis decreased IL-17 serum levels, pain and burning sensation severity (based on VAS) and the maximum of lesion diameters in patients with ulcerative oral lichen planus.

INTRODUCTION

Ulcerative oral lichen planus is a T lymphocyte disease with a chronic inflammatory nature, which does not respond well to routine treatments. Specific and non-specific antigenic mechanisms are involved in its pathogenesis. In the non-specific mechanism, a reciprocal reaction between mast cells and T cells results in the release of cytokines, chemokines and metalloproteinasmes from mast cells, which result in an increase in the activity, migration ad differentiation of T lymphocytes. (Kuo, R.C., et al., 2012)

Increased attention to the etiopathogenesis of immunologic and inflammatory conditions has highlighted the role of chemokines and cytokines. (Mesquita, D., et al., 2009)

Interleukin-17 family consists of cytokines which are secreted by T helper-17 cells and have an important role in the regulation of mucous and epithelial immune responses. (Pappu, R., et al., 2012) The role of IL-17 has been demonstrated in the etiology and pathogenesis of a number immunologic and inflammatory disease, including psoriasis (Leonardi, C., et al., 2012), rheumatoid arthritis (Xie, Q., Cunsh, li J., 2012), Behçet’s diseases (Liu, X., et al., 2011), multiple sclerosis (Pollinger, B., 2012), and recurrent aphthous stomatitis. (Lewkowicz, N., et al., 2011) On the other hand, in a number of animal and human studies, a definite and purposeful treatment strategy to neutralize or decrease IL-17 has resulted in attention to anti-IL-17 human

Several animal and in vitro studies have shown the preventive, anti-tumoral and antioxidative effects of propolis in relation to prevention of conversion of premalignant lesions to malignant lesions in laboratory animals and human cell lines in vitro. (Solaiman, G.H., et al, 2012; MohammadzadehSh, M. et al., 2007). The decreasing effect of propolis on interleukins, especially IL-17, has been reported in animal studies (Tanaka, M., et al., 2012) and human cell lines in vitro. (Szliszka, E., et al., 2013) Propolis has been used in dentistry in wound healing processes of the oral cavity and in controlling gingivitis. (Handa, A., et al., 2011) Based on data available, no studies have to date evaluated the effect of oral propolis in patients with ulcerative oral lichen planus. The present study was designed to evaluate the effect of oral propolis on the serum levels of IL-17 and the clinical signs of ulcerative oral lichen planus.

**MATERIALS AND METHODS**

The present clinical trial was carried out in the Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences. The study was registered in the Iranian Clinical Trial Center and was allocated the unique code of IRCT 201301011976N1. A total 27 patients (7 males and 20 females) were included in the study by simple sampling technique.

The minimum and maximum ages of the participants were 37 and 69 years, respectively. The condition was diagnosed clinically and histopathologically. None of the subjects in the present study had taken any corticosteroids or immunosuppressives during the previous 2 months to control oral lichen planus or other medical conditions. In addition, biopsies were not contraindicated for any of the subjects. None of the patients had lichenoid lesions due to contact with amalgam or drug lichenoid reactions.

Blood samples (5 mL) were taken at 9–11 in the morning from the cubitus veins and after isolation of the blood serum, the samples were stored at -80ºC. After the initial blood sampling procedure, each patient was given thirty 500-mg propolis capsules to be taken orally after breakfast with a glass of water for 30 days. The patients were instructed to take the capsules daily and regularly. Patients who did not take the capsules or exhibited allergic reactions or untoward drug reactions such as gastrointestinal problems were excluded from the study.

On the days 10 and 20 the patients were contacted and questioned in relation to the correct use of the medication, any side effects and the clinical status of each patient.

The propolis used in the present study was fresh green propolis produced from the Sabalan Mountain foots, near Ardebil, in the NW of Iran. The propolis was prepared in the Faculty of Pharmaceutics, using extraction technique by immering in ethanol in three steps. Each 500-mg capsule contained 325 mg of pure extract and 175 mg of neutral filing material.

After one month the patients were recalled and once again 5 mL of blood sample were taken. During the study one patient was excluded due to headache and another was excluded due to gastrointestinal disturbances (abdominal pain).

The special human kit for measuring IL-17 serum level (eBioscience, USA) and an ELISA equipment (Elisa Awareness, USA) were used to determine IL-17 serum levels. The assay was carried out according to the standard technique and based on the special instructions of the kit. At the end of the assay, the optical densities on the plates were read by the equipment at a wavelength of 450 nm and IL-17 serum levels were determined at weight scales in terms of pg/mL based on standard concentrations after drawing graphs for each optical density.

**Results:**

The means of IL-17 serum levels before and after treatment with propolis were 135±16.5 and 127±13 pg/mL, respectively, demonstrating a decrease in IL-17 serum levels after treatment with propolis. Normal distribution of data was evaluated by Kolmogorov-Smirnov test using SPSS 13, which revealed that data were parametric. Therefore, paired t-test was used to evaluate data, which demonstrated significant differences in IL-17 serum levels before and after treatment at a significant level of P<0.05.

In addition, the means of pain and burning sensation severity based on VAS before and after treatment with propolis were 4.2±3.6 and 3.3±2.8, respectively, indicating a decrease in pain and burning sensation severity after treatment with propolis, with statistically significant differences between the two periods, before and after treatment (P=0.049).

Wilcoxon’s test was used to evaluate the diameters of lesions because data were non-parametric. The means of lesion diameters before and after treatment were 14±7.7 and 9.8±7.6 mm, respectively, indicating a decrease
in the mean lesion diameter after treatment, with statistically significant differences between the two periods (P=0.03).

**Fig. 1:** The error bars of IL-17 serum levels.

**Fig. 2:** The error bars of pain severity before and after treatment with propolis.

**Fig. 3:** The error bars of lesion sizes before and after treatment with propolis.

**Discussion:** Based on data available, the present study is the first study on the use of a natural product (propolis) with the aim of decreasing IL-17 serum levels in patients with ulcerative oral lichen planus, given the etiopathogenesis of the disease, i.e. the role of proinflammatory cytokines. The role of proinflammatory cytokines has been shown in the etiology and pathogenesis of a large number of immunologic and inflammatory conditions. (Gaffen, S., 2008) In animal studies by Pollinger et al in 2012 the effective and intensifying role of IL-17 in laboratory rats with multiple sclerosis (MS) and rheumatoid arthritis was evaluated. (Pollinger, B., 2012) In a study by Rafa et al in 2013 the role of IL-23/IL-17 axis in inducing bowel inflammatory diseases and
the pathogenic and intensifying role of these cytokines in Crohn disease and ulcerative colitis was emphasized. (Rafa, H., et la., 2013)

In another animal study by Bosman et al in 2013 the role of catecholamines and glucocorticoids in controlling proinflammatory cytokines, including IL-17 family, in rats artificially afflicted with disseminated sepsis was evaluated and it was shown that when the adrenal gland is removed, rat mortality rate increases and the serum levels of IL-17 increase 3 to 10 folds. The study showed the important role of IL-17 in inflammatory diseases and also showed an increase in the serum levels of this cytokine in such diseases. (Bosmann, M., et al., 2013)

In a human study by Wang Hu et al in 2013 the reciprocal interaction between lichen planus and chronic periodontitis and serum levels of IL-17 was evaluated and the results showed significantly higher serum levels of IL-17 in patients with concomitant oral lichen planus and chronic periodontitis compared to the healthy controls. The highest serum levels of IL-17 were reported in female patients with concomitant ulcerative oral lichen planus and chronic periodontitis. The results also showed that an increase in the serum levels of IL-17 in the ulcerative type of lichen planus was higher than those with lower levels of inflammation, somehow demonstrating the proportionality of IL-17 and the inflammation severity. (Wang, H., et al., 2013)

One of the materials referred to in various animal and laboratory studies and a few human studies in relation to its antiinflammatory and antineoplastic effects is propolis.

In a study on human cell lines by Zukowska et al in 2013 the effective and antineoplastic role of propolis in glioblastoma cell lines was emphasized. (Zukowsk, R.M., et al., 2013) In an animal study by Okzyk et al in 2013 the regenerative and reconstructive effect of propolis on type I and type II collagen in burns artificially induced in pigs was evaluated and the results showed the positive effect of propolis on regeneration of these wounds. (Olczyk, P., et al., 2013) In an animal study by Tanaka et al in Japan in 2012 the effect of propolis on decreasing IL-17 serum levels was shown in rats experimentally afflicted with arthritis. Systemic administration of propolis in rats resulted in the improvement of clinical signs of arthritis in rats. (Tanaka, M., et al., 2012)

In an in vitro study on human cell lines by Szliszka et al in 2013 the effective role of propolis and its antiinflammatory and antioxidative effect and also its role in decreasing proinflammatory cytokines, such as IL-17, was shown on human cell lines. (Szliszka, E., et al., 2013) In the present study, too, the antiinflammatory properties of propolis and its effect on decreasing serum levels of IL-17 and on the clinical signs in patients with ulcerative oral lichen planus were shown.

In a human clinical trial by Samet et al in Britain in 2007 the effect of 500-mg oral capsules of propolis on the healing of recurrent aphthous stomatitis ulcers was evaluated and the results showed a decrease in the recurrence rate and an improvement in the patients’ quality of life. (Samet, N., et al., 2007) In another human study, the effect of anti-IL-17 (anti-IL-17 monoclonal antibody) on the healing of chronic cutaneous psoriasis plaques was evaluated and the results showed that anti-IL-17 human monoclonal antibody (Ixezikumb) improved the clinical symptoms and signs in patients with psoriasis. The study is important because of the similarities between psoriasis and its inflammatory and chronic nature to those of lichen planus and the results can be effective for the treatment of lichen planus. Hofstetter et al evaluated the role of IL-17 on rats with MS in 2009 and the results showed that T helper-17 cells can be considered a cell population effective in the pathogenesis of MS. Therefore, adoption of a definitive purposeful treatment strategy to neutralize IL-17 can be a promising and new treatment modality for this condition. (Hofstetter, H.H., et al., 2009)

Considering the results of studies mentioned above it appears the serum levels of interleukins, especially IL-17, increase in a wide range of inflammatory and immunologic conditions. Therefore, finding techniques and materials that can control and decrease IL-17 serum levels can open new horizons for the treatment of such medical conditions. No side effects have been reported for the oral administration of propolis and the majority of articles available have reported a limited number of contact allergic reactions and a few cases of generalized dermatitis. In some reports, too, a few cases of gastrointestinal disorders have been mentioned. Budmir et al reported a case of perioral dermatitis and contact cheilitis in an 18-year-old female patient who had used propolispray. (Budmir, V., et al., 2012) Cho et al reported a case of generalized dermatitis in a 36-year-old female patient who had practiced self-treatment by taking propolis for a few weeks. (Cho, E., et al., 2011)

In the present study, two subjects were excluded from the study; one of the subjects developed headaches after taking propolis capsules but recovered after discontinuing the medicine and re-developed headaches after taking the medicine again. The other patient developed abdominal pain after taking propolis capsules. These two patients received routine treatment for lichen planus. Headaches have not been reported as a side effect for propolis and they might be considered one of the side effects for propolis.

In a human clinical trial by Hueber et al in 2012 the effect of scukinnab which is an anti-IL-17 monoclonal antibody was evaluated in patients with moderate-to-severe Crohn disease and no significant clinical results were reported. (Hueber, W., et al., 2012) Based on data available no studies to date have shown a significant decrease in IL-17 levels in inflammatory or immunologic conditions. In addition, no reports are available on the effect of propolis on increasing the serum levels of IL-17 in animal, in vitro or human studies.
From the results of previous studies and those of the present study, which is the first human study on the effect of propolis on interleukin serum levels and clinical signs of ulcerative lichen planus, it can be concluded that propolis, a natural product, has a decreasing effect on IL-17 levels, which has a background role in inducing lichen planus; therefore, propolis might be considered a medication, which apart from its symptomatic effects, can have an effect on the main course of ulcerative oral lichen planus.

The importance of this consideration is obvious when it is noted that there are no known cures for oral lichen planus with minimum side effects. However, more studies are necessary to further evaluate the effect of propolis on the ulcerative oral lichen planus itself.

Conclusion:
Oral administration of propolis in patients with ulcerative oral lichen planus results in a significant decrease in IL-17 serum levels and an improvement in clinical symptoms and signs (a decrease in pain severity based on VAS and a decrease in wound diameters) compared to the period before treatment.

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REFERENCES


Pollinger, B., 2012. IL-17 Producing T cell in mouse models of multiple sclerosis and Rheumatoid arthritis. J of Molecular medicine, 10: 841-44.


