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Effect of Berberine Gel on Periodontal Inflammation: Clinical and Histological

Amir Moein Taghavi[1,2]* • Malihe Shabzendedar[1,3] • Iman Parissay[4] • Abbas Makarem[1,5] • Hossein Orafaei[6] • Mona Hosseinnezhad[7]

[1]Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Associate Professor, Department of Periodontics, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Assisstant Professor, Department of Periodontics, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Assisstant Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Yazd University of Medical Sciences, Yazd, Iran
[5]Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Associate Professor, Facutly of Pharmacology, Mashhad University of Medical Sciences, Mashhad, Iran
[7]Periodontist, Private Practice, Mashhad, Iran
*Corresponding Author; E-mail: moeentaghavia@mums.ac.ir

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Abstract

Background and aims. Previous data have demonstrated that berberine, a barberry plant alkaloid, possesses various therapeutic properties. This study histologically evaluated the clinical efficacy of a berberine-derived topical gel on periodontal inflammation in patients requiring surgery.

Materials and methods. This randomized clinical trial was performed on 14 patient (11 females, 3 males) presenting with moderate to severe periodontitis (pocket depth >4 mm). Plaque indices (PI) and gingival indices (GI) were recorded at baseline and scaling and root planing were carried out. One week later, PI and GI values were recorded again in patients treated on respective sides of the jaw with either berberine gel or a placebo gel control at night for two weeks. PI and GI were again measured prior to periodontal surgery (three weeks after the initial visit). Specimens collected from both sides of the jaw were evaluated histologically and PI and GI scores compared for statistical differences.

Results. GI and PI scores were significantly different between baseline and follow-up examinations but no significant differences were observed between the groups. No differences in the nature of the inflammatory cell types, degree of angiogenesis, integrity of collagen fibers and the levels of edema were observed between the groups; however, barberry gel-treated tissues presented with reduced levels of inflammatory cell infiltrates compared to placebo controls.

Conclusion. Tissues treated with barberry gel extract had reduced numbers of inflammatory cells at the time of surgery. However, the GI and PI scores were not significantly different between the groups.

Key words: **Barberry bush extract, gingivitis, inflammation, periodontitis.**

Introduction

Gingivitis and periodontitis are the most common oral inflammatory diseases. Gingivitis involves primarily inflammation of gingival tissues and periodontitis is inflammation of the tissues and ligaments that support teeth, leading to tooth mobility and subsequent tooth loss. It is caused by infections with microorganisms present in the dental plaque.[1] Periodontal inflammation typically consists of increased leukocyte infiltration and changes in vascular permeability.[1]

Chemical plaque control and prevention have been the focus of various periodontal preventive strategies since 1980 with the use of antibiotics (e.g. tetracyclines and metronidazole), enzymes (e.g. dextranase mutans), antiseptics (e.g. chlorhexidine), quaternary ammonium compounds (e.g. cetylpyridinium), phenols, oils, and herbal compounds.[2] Novel therapies include the use herbal-based pharmaceutical products that have been used worldwide, including the increased use of herbal toothpastes over the last decade in the United States because many consumers believe that herbal-based products are often safer and more effective than chemical-based products.[3]

A key to selecting pharmaceutical plant derivatives for use in medicinal preparations is their global availability. Barberry bush preparations have a wide range of pharmacological effects, including anti-inflammatory properties.[4,5] This plant can be grown in Europe, Africa, the Americas and in the central Asia. It is found exclusively in Iran. The barberry root and the stem have antiseptic properties and the stem, root and bark have also been used to treat digestive disorders of septic origin, chronic dyspepsia with loss of appetite, gastric cramps, rheumatism or septic and biliary fevers. The brewed barberry root bark and stem have also been recommended for the treatment of gastric problems, as a component of eye rinse solutions and as a mouthwash component used in caries prevention.[6,7]

Biochemical studies have shown that the constituents of this plant include isoquinoline alkaloids, phenolic compounds and triterpenoids. Extracts prepared from this plant have conferred anti-arryhthmic, anti-babesial**,** anti-cholinergic, anti-histamine, anti-hypertensive, anti-inflammatory and anti-nociceptive properties in addition to vasodilatory effects.[8] Furthermore, studies have demonstrated that alkaloids such as berberine were more effective against dental bacteria such as *A. actinomycetemcomitans* and *P. gingivalis* than against lactobacilli and streptococci.[9] Berberine also inhibited collagenase activity of *A. actinomycetemcomitans* and *P. gingivalis*, two major periodontal pathogens.[9]

Various studies have shown increased, worldwide gingivitis prevalence rates, especially in developing countries. For example, only 11.3% of 15−19 year-old Iranians had healthy periodontal tissues, 12% had bleeding on probing, 46% presented with gingival calculus, 30.4% had shallow pockets and 0.3% had deep pockets in their jaw sextants.[10]

Makarem et al[11] concluded in 2005 that a dental gel preparation containing berberine reduced dental plaques by 56% and their study resulted in a 33% improvement in the GI. This study was performed to evaluate the clinical and histological efficacy of a topical gel containing a barberry extract in patients with periodontitis needing periodontal surgery.

Materials and Methods

Sample Size

Based on the study by Makarem et al[11] with α=0.05 and β=0.2, eleven patients were the minimum needed to carry out the proposed study; however, 14 patients were recruited to accommodate confounders.

Study Design

This randomized clinical trial was performed on 14 patients (11 females, 3 males) with a mean age of 45±4 years, who had referred to the Department of Periodontology at Mashhad Faculty of Dentistry, Iran. All the patients presented with moderate to severe periodontitis according to criteria established by the American Academy of Periodontology (AAP) and needed periodontal surgery (pocket depth >4 mm).

The study protocol was approved by the Medical Ethics Committee of Mashhad University of Medical Sciences and registered at www.irct.ir. Subsequent to receiving information regarding the study process informed consent was obtained. Patients with the following conditions were excluded from the study: patients with conditions that could aggravate periodontal infections (such as hematologic disorders, diabetes, immunodeficiencies), antibiotic use during the preceding three-month period, patients on contraceptives, patients using antibacterial mouthwashes or patients with a history of smoking.

Gel Preparation

Berberis vulgaris branches were collected in the autumn and dried outdoors for three weeks. The degree of dehydration was verified periodically by measuring the weight of the collected branches. The branches were ground to a particle size of 1000±250 μ; 200 g of the ground particles were extracted fol-

lowing the reflux protocol over a 24-hour period with 700 mL of 96% ethanol using Soxhlet instrument. The alcohol extract was concentrated to yield 40 g using vacuum evaporation at 40°C water bath. The extract was standardized using UV spectroscopy at 340 nm on the basis of the berberine concentration of the primary plant alkaloid that comprised 0.005% of the total dried branch weight. The 5% aqueous gel specimens were prepared by geometrically triturating 5 g of the extract with 95 g of gel base under clean conditions using a mortar and a pestle. The gel base was an aqueous solution of 5% polyvinyl alcohol. The placebo gel was prepared in a similar fashion except for the incorporation of the concentrated berberine extract. Fifteen grams of either the berberine gel preparation or the placebo were packed in aluminum tubes on the same day of delivering to the patients under clean conditions.

Berberine Gel Testing

Baseline PI and GI indices of enrolled patients were recorded at the time of the study (baseline) and again one-week later. In addition, scaling and root planing were carried out for all the patients using an ultrasonic scaler (Dentsply, Cavitron; BOBCAT, 11136, L.I city, N.Y, USA) following standard protocols. An impression was taken of the jaw and a soft splint was made with a medial gap. The patients were asked to fill half of the splint with berberine gel and half with placebo each night for a period of two weeks at which time the PI and GI were again measured prior to surgery. To allow for patient use errors, each patient received two coded tubes containing berberine gel or placebo. The patients were asked to return the tubes after two weeks and the content of the respective tubes was identified when the patients returned to the clinic at the end of the two-week period.

Three weeks after scaling and root planing, periodontal surgery was performed and gingival specimens from interdental papilla were harvested from both sides of the jaw and analyzed histologically. Samples were fixed in 10% formalin for 24 hours, paraffin embedded, cut into 4−5-µm-thick sections, stained with hematoxylin and eosin (H&E) and examined under ×400 and ×1000 magnifications using a light microscope (Leitzlabarlux Microscope, Vermont Optechs, Charlotte, VT).

Histological Evaluation

Acute and chronic inflammation was defined by characterizing the nature of infiltrating polymorphonuclear (PMN) cells and lymphocytes. The severity of inflammation was categorized according to the number of inflammatory cells present in the respective microscopic fields. Degrees of inflammation were defined as follows:

- 0−2 inflammatory cells, no inflammation
- 2−5 inflammatory cells, mild inflammation
- 5−10 inflammatory cells, moderate inflammation
- 10 or more inflammatory cells, severe inflammation

The number of blood vessels in 5 microscopic fields (0.2 mm^2) was calculated and compared to the number of blood vessels present in samples harvested from control specimens. In addition, changes in epithelial thickness were compared to epithelial thickness of normal tissues and the results were defined as either hyperplastic or atrophic. The examiner, surgeon and statistician were all blinded to the medication applied to respective samples. Two patients were excluded due to non-compliance.

Statistical Method

Gingival and plaque indices for the two groups were analyzed using the Friedman test. Chi-squared and Wilcoxon tests were used to compare inflammation rates and vessel densities between the groups.

Results

Fourteen patients were enrolled in this study to assess the effect of berberine gel on periodontal inflammation. Two patients were excluded due to non-compliance. Of the 12 remaining patients (2 men, 10 women) differences in respective GI values were observed between baseline and follow-up visits in each group (Tables 1 & 2); however, no GI differences between the respective groups at each time interval were observed although PI decreased sig-

Table 1. Gingival index reading of test and control groups at each visit

Visit	Test Mean ± SD	Control Mean ± SD	P value*
First	1.57±0.43	1.68±0.4	0.214
Second	1.37±0.26	1.28±0.4	0.386
Third	1.07±0.42	1.09±0.29	0.779
P value**	0.037	0.002	

*Wilcoxon's signed rank test
**Friedman test

Table 2. Plaque index readings of test and control groups at each visit

Visit	Test Mean ± SD	Control Mean ± SD	P value*
First	1.82±0.43	1.72±0.77	0.114
Second	1.46±059	1.42±0.61	0.715
Third	1.04±0.8	1.1±0.75	0.068
P value **	0.013	0.019	

*Wilcoxon's signed rank test
**Friedman test

nificantly between the first and third visits. The most commonly identified inflammatory cell types in respective samples were lymphocytes and plasma cells. However, there were no significant differences in the type of inflammatory cells between the groups, the degree of angiogenesis (P=0.102) and the degree of edema (P=0.214) between samples from the respective treatment groups. In addition, the amount of collagen fibers remained unchanged between the groups. According to Table 3 the only significant difference observed was a decrease in the number of inflammatory cells in samples examined from portions of the jaw treated with berberine gel (P=0.011).

Discussion

Gingivitis and periodontitis are two common inflammatory diseases of periodontal tissues. Inflammation is limited to gingival tissues in gingivitis but periodontitis is associated with the destruction of tooth supporting structures. In both cases, inflammation is the result of microorganisms present in dental plaque.[1] Therefore, mechanical or chemical plaque control methods have been used to reduce plaque-related inflammation of the oral mucosa.

Since it has been suggested that there is an association between periodontal disease and systemic diseases like coronary heart diseases, diabetes, stroke or preterm low birth weights, control of periodontal infections could be important not only in controlling oral mucosa infections but also in the maintenance of overall health.

Today, chemical plaque control using mechanical methods has increased the efficacy of periodontal treatments along with antibiotic treatments and essential oils used for plaque control.[2] Since herbal derivatives are less harmful than synthetic medications tremendous efforts have been made to identify novel herbal extracts for use as anti-plaque agents.[3]

Barberry is a plant that grows in different parts of the world, including parts of Europe, Africa and in Asia. It is an indigenous plant in Iran.[11] Berberine is the most effective alkaloid derived from barberry plants and has been added to toothpastes and mouthwashes due to its antimicrobial activities. Since it was demonstrated by Makarem et al[11] that berberine gel reduces both PI and GI in gingivitis patients, the present study was carried out to evaluate the clinical and histological effects of berberine gel in periodontitis patients. The results showed that PI and GI decreased significantly between baseline and the second and third visits in both groups, possibly due to mechanical debridement, in contrast to the data presented by Makarem et al[11] suggesting that the reduction in both PI and GI was due to the berberine present in the toothpaste and not a consequence of mechanical debridement. To eliminate the mechanical debridement component from this study, the patients in the present study used the gel during their sleep and not during tooth cleaning.

Data from studies that required patients to carry out some of the study procedures at home demonstrated that patients might be influenced by factors that may mask the efficacy of a test agent compared to the control. One factor is the Hawthorn effect that suggests that clinical trial participants may experience some improvements not associated with the therapeutic properties of the test agent, but rather due to behavioral modifications as a consequence of participating in the trial.[12] For example, patients participating in oral hygiene studies improved their oral care practices regardless of the group in which they were enrolled. Since this study had a split-mouth design and each patient acted as a test and a control, risk of Hawthorne effect bias decreased. However, it is possible that the 3-week period of the study was insufficient to show significant effects of berberine gel in comparison to the placebo due to a potential lack of compliance with the gel use.

Although it has been demonstrated that berberine and related derivatives (such as oxycanthine) possess antibacterial properties[13] and can inhibit bacterial attachment to human cells,[14] no significant effects of berberine were observed compared to placebo except for the intensity of the inflammatory cell infiltrate which decreased in berberine-treated tissues. This might be attributed to the anti-inflammatory effects of berberine and berbamine, another alkaloid shown to improve immune cell function.[15,16]

Table 3. Inflammatory cell infiltrate intensity

	Test		Control		
Intensity	**Number**	**Percent**	**Number**	**Percent**	**P value**
Mild	5	41.7	0	0	
Moderate	5	41.7	7	58.3	
Severe	2	16.7	5	41.7	
Total	12	100	12	100	P=0.011

Conclusion

The use of a barberry-derived gel, compared to the placebo, did not alter GI or PI scores, inflammatory cell profiles or the severity of edema but reduced the degree of inflammatory cell infiltrates in the oral mucosa.

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