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Clinical and Histological Evaluation of Barberry Gel on Periodontal Inflammation

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1. Introduction

Gingivitis and periodontitis are the most common inflammatory oral diseases (Newman et.al./2006). Gingivitis is the most common periodontal disease in children and adolescents (Mc Donald et.al./2011). Periodontitis is an inflammation of the tissues and ligaments that support teeth (that could lead to loosening and subsequent tooth loss) due to infections with microorganisms participate in dental plaque (Newman et.al./2006).

Gingivitis involves primarily inflammation of gingival tissues (Newman et.al./2006). Clinically, it appears as an inflammation of the gingival tissues next to the tooth. Microscopically, it is characterized by the presence of an inflammatory exudates and edema, destruction of collagenous gingival fibers, and ulceration and proliferation of the epithelium facing the tooth and attaching the gingival to it (Mc Donald et.al./2011).

Various studies have shown increased, world-wide 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 during probing, 46% presented with gingival calculus, 30.4% had shallow dental pockets and 0.3% had deep pockets in their jaw sextants (Kazemnejad et. al./ 2008, Khordimood & Makarem /2002).

Despite of the changing concepts on the etiology that considered plaque as an etiologic factor for periodontal diseases, an oral self care (for plaque control) is still an essential step in the prevention and treatment of gingivitis (Marsh &Bradshaw1993.Makarem et.al.2006). Bacterial plaque is composed of soft bacterial deposits that adhere firmly to the teeth and form a complex, metabolically interconnected, highly organized bacterial system consisting of dense masses of microorganisms embedded in an inter microbial matrix. *In sufficient concentration, this microbial matrix can disturb the host-parasite relationship and cause dental caries and periodontal diseases (Mc Donald et.al./2011).*

Since most individuals, especially children and adolescents seem to have difficulty in achieving perfect plaque control by mechanical means, investigations have been directed towards the dentifrices (Pooreslami & Makarem /2002).

2. Plaque control

Chemical plaque control and prevention has been focused on various periodontal preventive strategies since 1980 and include the use of antibiotics (e.g., metronidazole),

enzymes (e.g., dextranase), antiseptics (e.g., chlorhexidine), quaternary ammonium compounds (e.g., cetylpyridinium), phenols, oils, and herbal compounds (Mc Donald et.al./2004).

Recently, novel therapies have included the use of herbal-based pharmaceutical products that have been used world wide, including the increased use of herbal toothpaste over the last decade in the United States, since the perception of many consumers is that herbal-based products are often safer and more effective than chemical-based products (Sean Lee, et.al./2004). Makarem, Khordimood & Pooreslami have shown that herbal agents have effective antiplaque characteristics which make them appropriate as possible antiplaque and tooth cleansing agents (Pooreslami & Makarem /2002 Khordimood & Makarem /2002).

Pradeep AR, et al. concluded that Gumtone gel may be a useful herbal formulation for chemical plaque control agent and improvement in plaque and gingival status (Pradeep /2010).

Adamkova H et al performed a clinical trial to investigate the effectiveness of a herbal-based dentifrice in the control of gingivitis. Forty volunteers were participated in a 84-days study. All subjects were balanced for measured parameters – plaque index (PI), community periodontal index of treatment needs (CPITN) and papillary bleeding index (PBI). The dentifrice was effective in reducing symptoms of gingivitis as evaluated by the CPITN and PBI indices (Adamkova).

Berberine is an alkaloid agent which has previously shown high antimicrobial effects (Makarem & Khalili/2006). This alkaloid is the most active alkaloid (isoquinolines group) extracted from the root and stem of the plant barberry which grows in Europe, Africa, America and central Asia and also in Iran (Makarem & Khalili /2006).

Its scientifically name is "Berberis Vulgaris", the herb known as "Barberry" is a thorny shrub with yellow flowers, small red fruits. It grows along with other shrubs at the edge of fields or forests. This herb is a little pretentious regarding the type of soil it grows on; the types of barberry with caducous leaves are heliophile, and those with persistent leaves can be cultivated in the shade.

In the traditional Chinese medicine, barberry has been mentioned for the past 3000 years. Barbarry is known to contain the potent active agent Berberine, which has numerous usages in controlling different illnesses (stimulates digestion and reduces the gastrointestinal pains). It is also known as a substance that toughens the immune system. Apart from berberine, there are numerous active substances present in the different parts of plant. The bark contains a large number of alkaloids (berberine, berbarine, oxyacantha) and tanines. Barberry fruits contain glucose, fructose, malic acid, pectin, vitamin C. The active substances from the herb bring about the following effects: haemostatic, diuretic, vasodilator, hypertensive, antibacterial, and anti-inflammatory.

Only the dry crust from the roots and stem is being used in medicinal purposes. Barberry can be found on the market under the forms of tea, tincture, pills and ointment. Usually the percentage of berberine from those products is between 8 and 12%. The tincture should be consumed three times a day in doses of 1.2 ml.

Barberry decoct as gargling is effective against sore throats while using of cataplasms with powdered barberry crust is recommended for conjunctivitis.

Anti-inflammatory

The effects of the alkaloid constituents are primarily responsible for the historical use of Berberidaceae species extracts in inflammatory conditions. Berberine and oxycanthine

alkaloids from *Berberis vulgaris* were administered in acute inflammation (paw edema). In comparison, Oxycanthine was less effective than berberine in the studies (Ivanovska & Philipov /1996).
An in vivo study using Turkish Berberis species demonstrated that all alkaloids (from this species of *Berberis*) inhibited inflammation with dose dependent activity. Berberine, palmatine and berbamine were the most effective in topical and oral administration.

3. History

Barberry has a long history of use in traditional eastern and western herbalism. In ancient Egypt, barberry fruit was used with fennel seeds to ward off pestilent fevers (Chevallier / 2001). Indian ayurvedic physicians used barberry in the treatment of dysentery and traditional Iranian medicine uses the fruit as a sedative (Kunwar et.al./2006, Fatehi-Hassanabad.al/2005). In northern Europe barberry was used to treat gall bladder and liver problems, while in Russia and Bulgaria it was used in the treatment of abnormal uterine bleeds and rheumatism (Ivanovska &, Philipov/ 1996, Imanshahidi & Hosseinzadeh/ 2008). In North America, the Eclectics used barberry to treat malaria and as a general tonic (Mills & Bone / 2000). The American Indians found it useful in improving appetite and used the dried fruit as a gargle (Imanshahidi & Hosseinzadeh /2008, Bone / 2003).

4. Major active constituents

The key active constituents of barberry root and stem bark are isoquinoline alkaloids. Two classes of alkaloids have been identified – protoberberines (berberine, berbamine, jateorrhizine and palmatine) and bisbenzlisoquinolines (oxycanthine). Berberine is the main active constituent and the most studied alkaloid. It is found throughout the plant; however, it is more concentrated in the roots, bark and stems (Imanshahidi & Hosseinzadeh/2008, Bone /2003).

5. Actions

5.1 Traditional

antimalarial, antirheumatic, antiseptic (Imanshahidi & Hosseinzadeh /2008, astringent Tierra/*1988*), bitter tonic (Imanshahidi & Hosseinzadeh /2008), depurative (Tierra/*1988*), diuretic (Ivanovska & Philipov /1996), dysmenorrhea (Imanshahidi M & Hosseinzadeh /2008), purgative (Mills & Bone/2000, sedative (Fatehi-Hassanabad et.al./2005).

5.2 Contemporary

amoebicidal , antibacterial (Chevallier / 2001), antibiotic (Van Wyk &, Wink /2004), antiemetic (Thomsen / 2005, Bone / 2007), anti-inflammatory (Thomsen / 2005), antimicrobial, antiparasitic (Bone / 2007), antiprotozoic , antipyretic (Thomsen/2005), bitter tonic (Kunwar et.al./2006,Van Wyk &,Wink /2004, Bone /2007), cholagogue (Thomsen /2005, Bone /2007, choleretic (Van Wyk &, Wink /2004), hepatic (Bone / 2007), laxative (Bone /2003, Bone /2007), spleen tonic (Thomsen/2005).
The Makarem and co workers study (Makarem & Khalili /2006) indicates that the barberry dental gel effectively controls microbial plaque and gingivitis in the school aged children; therefore, the use of barberry dental gel is strongly recommended. They also concluded that a dental gel preparation containing berberine reduced dental plaques by 56% and their study resulted in a 33% improvement in the GI (Makarem & Khalili /2006).

The study of Moeintaghavi , Makarem et al, was performed to evaluate the clinical and histological efficacy of a topical gel containing a barberry extracts in patients with periodontitis needing periodontal surgery. They concluded that 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 different between treated groups.

6. The study protocol

6.1 Sample size

Based on the study by Makarem *et al*., 11 patients were the minimum needed to carry out the proposed study; however, 14 patients were recruited to account for confounders.

6.2 Study design

This randomized clinical trial study was performed on 14 patients (11 female, 3 male) with a mean age 45±4 years that were referred to the Department of Periodontology at the Mashhad School of Dentistry, Iran. All patients presented with moderate to severe periodontitis according to criteria established by the American Academy of Periodontology (AAP) and also needed periodontal surgery.

The study protocol was approved by the Medical Ethics Committee of Mashhad University of Medical Sciences. 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 months, patients on contraceptives, patients using antibacterial mouthwashes or patients with a history of smoking.

6.3 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. A total of 200 g of the ground was extracted following the reflux protocol over a 24 h period with 700 ml of 96% ethanol using Soxhlet instrument. The alcohol extract was concentrated to give 40 gm using vacuum evaporating at 40°C water bath. The extract was standardized using UV spectroscopy at 340 nm on the basis of the berberin concentration of the primary plant alkaloid that comprises 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 mortar and pestle. The gel base was an aqueous solution of 5% polyvinyl alcohol. The placebo gel was prepared in a same manner without addition of the concentrated berberin extract. Fifteen grams of either the berberin gel preparation or the placebo were packed in aluminum tubes on the same day of delivering to the patients under clean conditions.

6.4 Berberin gel testing

Plaque (PI) and gingival (GI) indices of enrolled patients were recorded at the time of the study (base line) and again one-week later. In addition, scaling and root planning were carried out for all 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 made with a medial gap. Patients were asked to fill half of the splint with b erberine 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 control for patient use errors, each patient received two coded tubes containing berberine gel or placebo. Patients were asked to return the tubes after the two weeks and the content of the respective tubes identified when the patients returned to the clinic at the end of the two-week period.
Three weeks after scaling and root planning, periodontal surgery was performed and specimens harvested from both sides of the jaw and analyzed histologically. Samples were fixed in 10% formalin for 24 h, paraffin embedded and cut into 4-5 μm thick sections that were hematoxylin and eosin (H&E) stained and then examined at 400 and 1000X using an optical microscope (Leitzlabarlux microscope, Vermont Optechs, Charlotte, VT).

6.5 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 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 and 10 or more inflammatory cells, severe inflammation. The number of blood vessels identified in 5 microscopic fields (0.2 mm^2) was calculated and compared to the number of blood vessels present in samples harvested from the control specimens. In addition, changes in epithelial thickness were compared to epithelial thickness of normal tissues and results 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.

6.6 Statistics

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

7. Results

Fourteen patients were enrolled in this study to assess the effect of berberin 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 (Table 1 and 2), however, no GI differences between the respective groups at each time point were observed even though the PI decreased significantly between the first and third visits. The most commonly identified inflammatory cell type in respective samples were lymphocytes and plasma cells. However, no significant differences in the type of inflammatory cells present between treatment groups, the degree of angiogenesis (P=0.102) nor in the degree of edema (P=0.214) was observed between samples from respective treatment groups. In addition, the amount of collagen fibers identified remained unchanged between groups. The only significant difference observed was a reduction in the number of inflammatory cells

present in samples examined from portions of the jaw treated with berberin gel (P=0.011) (Figs 1-4).

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 signed Rank Test
**Friedman Test

Table 1. Gingival Index reading of the 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 signed Rank Test
**Friedman Test

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

Intensity	Test		Control		P-value
	Number (n)	Percent (%)	Number (n)	Percent (%)	
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

Table 3. Inflammatory cell infiltrate intensity

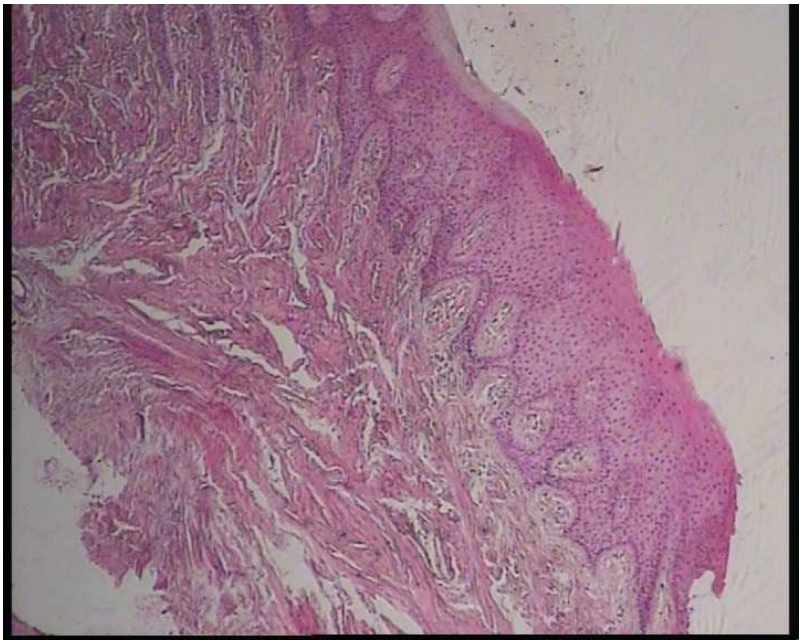

Fig. 1. Histologic aspect of a test specimen (H & E staining, magnification 40X)

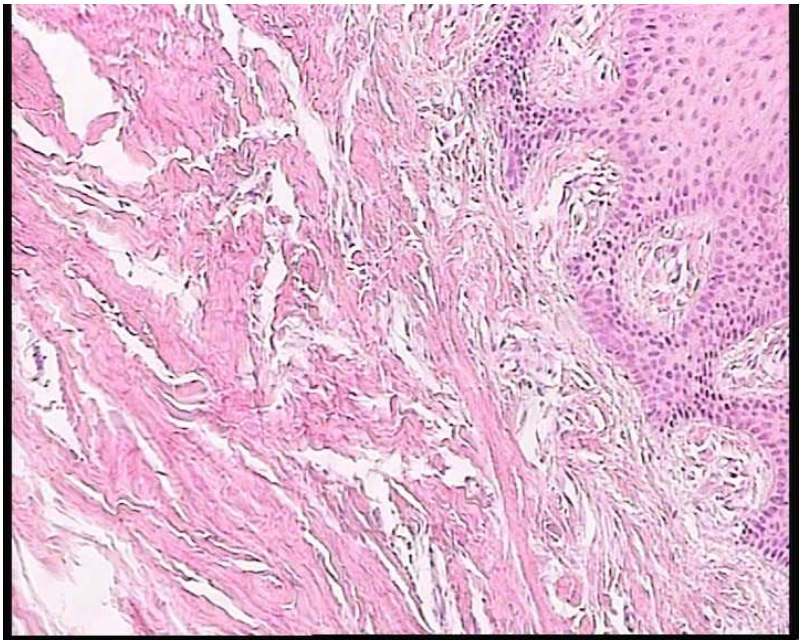

Fig. 2. Histological aspect of a test specimen (H & E staining, magnification 100X)

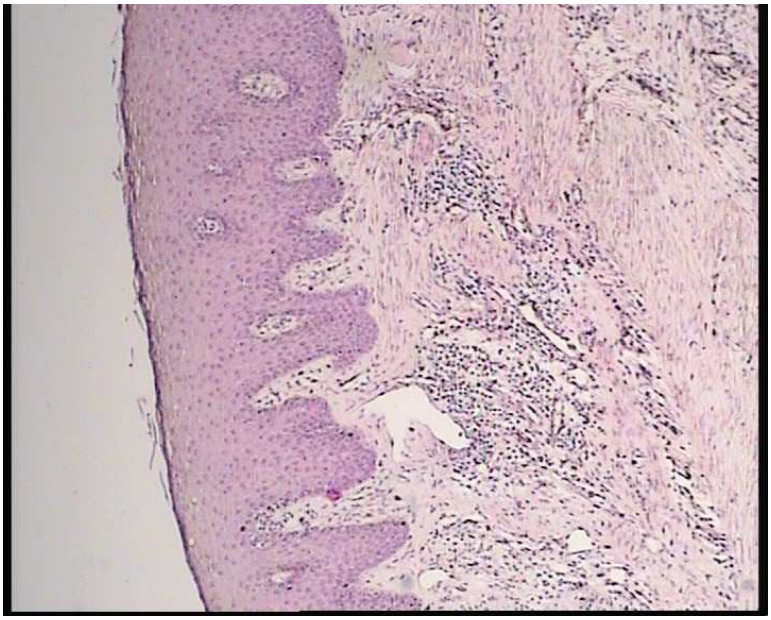

Fig. 3. Histologic aspect of a control specimen with chronic inflammation (H & E staining, magnification 40^{X})

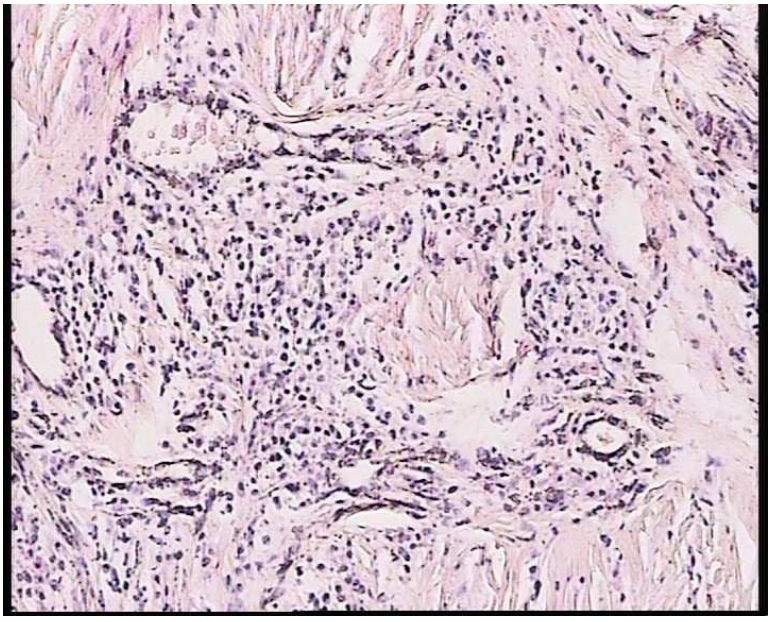

Fig. 4. Histological aspect of a control specimen with chronic inflammatory cells infiltration (H & E staining, magnification 100^{X})

8. Discussion

Gingivitis and periodontitis are two common inflammatory diseases of periodontal tissues. Inflammation is limited to gingival tissues in gingivitis but periodontitis is also associated with the destruction of tooth supporting structures. In both cases, inflammation is the result of microorganisms present in dental plaque (Newman et. al. /2006). Therefore, either 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 (Mc Donald et.al./2004). Since herbal derivatives are less harmful than synthetic medications (Lee et.al./2004) significant efforts have been made to identify novel, herbal extracts for use as anti-plaque agents.

Barberry is a plant that grows in different parts of the world including parts of Europe, Africa and in Asia it is found only in Iran (Makarem & Khalili /2006). Berberin is the most effective alkaloid derived from Barberry plants and has been added to tooth pastes and mouth washes due to its antimicrobial activities. Since it was demonstrated by Makarem et al. that berberin gel reduced both the PI and GI in gingivitis patients (Makarem & Khalili /2006), the present study was carried out to evaluate the clinical and histological effects of berberin gel in periodontitis patients. Our findings showed that PI and GI decreased significantly between baseline and the second and third visits in both groups, likely due to mechanical debriment, in contrast to the data presented by Makarem et al. (Makarem & Khalili /2006) that suggested the reduction in both PI and GI was due to the berberin present in the toothpaste and not a consequence of mechanical debriment. To eliminate the mechanical debridment component from this study, our patients used the gel during their sleep and not during teeth cleaning.

Data from studies that required patients to carry out some of the study procedures at home demonstrated that patients could be influenced by factors that may mask the efficacy of a test agent compared to the control. One factor is the Hawthorn effect (Fletcher et.al/1997) that suggests the clinical trial participants may experience some improvement not associated with the therapeutic properties of the test agent, but rather due to behavioral modifications as a consequence of participating in the trial. 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 was reduced. However, it is possible that the 3-week period of the study was insufficient to show significant effects of the berberin gel over the placebo due to a potential lack of compliance with using the gel.

Although, it has been demonstrated that berberine and related derivatives (such as oxycanthine) poses antibacterial properties (Amin AH et. al./1969) and can inhibit bacterial attachment to human cells (Sun/ 1988) we did not observe significant effects of berberin over placebo with the exception of the intensity of the inflammatory cell infiltrate which was reduced in berberin-treated tissues. This might have been due to the anti inflammatory

effects of berberin and berbamine (Wong et. al. / 1992), other alkaloids shown to improve immune cell function (Kumazawa et. al. /1984).

9. 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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11. Conflict of interest and sources of funding statement

The authors declare that there were no conflicts of interest in this study.
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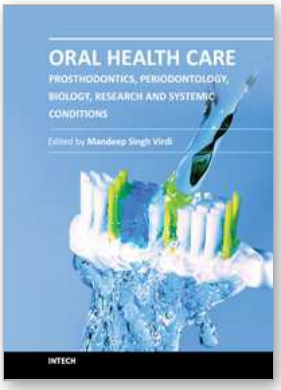

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Geriatric dentistry, or gerodontics, is the branch of dental care dealing with older adults involving the diagnosis, prevention, and treatment of problems associated with normal aging and age-related diseases as part of an interdisciplinary team with other healthcare professionals. Prosthodontics is the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation, and maintenance of the oral function, comfort, appearance, and health of patients with clinical conditions associated with missing or deficient teeth and/or oral and maxillofacial tissues using biocompatible materials. Periodontology, or Periodontics, is the specialty of oral healthcare that concerns supporting structures of teeth, diseases, and conditions that affect them. The supporting tissues are known as the periodontium, which includes the gingiva (gums), alveolar bone, cementum, and the periodontal ligament. Oral biology deals with the microbiota and their interaction within the oral region. Research in oral health and systemic conditions concerns the effect of various systemic conditions on the oral cavity and conversely helps to diagnose various systemic conditions.

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