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Interleukin-10 and Interleukin-1 β Cytokines Expression in Leukocytes of Patients with Chronic Peri-Mucositis

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Background: The purpose of the present research is to analyze the effect of polyphenols and flavonoids substrat (PFS) from plants *Calendula officinalis*, *Salvia fruticosa*, *Achillea millefolium*, and propolis as immunomodulatory in the production of interleukin (IL)-1 β and IL-10 in peripheral blood leukocytes medium (PBLM) in patients who were diagnosed with mucositis of peri-implant tissue compared to patients with healthy implant tissue. It was hypothesized that IL-1 β and IL-10 contribute to the inflammation processes noticed in the diseases of peri-implant tissues.





Material/Methods: Sixty non-smoking patients were included in this study: patients with healthy implants (HP group) and patients with peri-implant mucositis (MP group). Peri-mucositis was diagnosed by radiologic and clinical examination. The PBLM from MP were treated with PFS at various concentrations. The levels of IL-10 and IL-1 β excreted by the PBLM stimulated and unstimulated with viable *Porphyromonas gingivalis* test-tube were committed by the enzyme amplified immunoassay sensitivity method.

Results: Unstimulated and stimulated PBLM and treatment with 5.0 mg/mL or 10.0 mg/mL of PFS in the MP group produced significantly higher levels IL-10 ($P < 0.001$) that analogous mediums of the HP group. The levels of IL-1 β decreased more considerably in the stimulated PBLM of the MP group than in those of HP group ($P < 0.001$) after the treatment with PFS at only 10.0 mg/mL concentration.

Conclusions: Theses results suggest that the solution of PFS might offer a new potential for the development of a new therapeutic path to prevent and treat peri-implant mucositis.

MeSH Keywords: **Interleukin-10 • Interleukin-1beta • Leukocytes • Mucositis**

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Background

Peri-implant disease is a chronic destructive inflammation around dental implants which is caused by bacterial infections and it can be classified into 2 stages. The first stage is peri-implant mucositis and the second stage is peri-implantitis. The prevalence of these diseases varies from 19% to 65% and from 1% to 47%, respectively, according to case definitions [1–3]. The supporting structures around implants could become affected by an inflammatory process of microbial origin [2]. Even though bacterial plaque is considered the primary etiological factor of peri-implant diseases, the volume of the inflammatory reaction to bacterial products includes lipopolysaccharides, endotoxins, and generated cytokines that are released by host inflammatory cells [4]. Host cells respond to bacterial challenge by releasing certain pro- and anti-inflammatory mediators, which in turn mediate degradation of collagen and extracellular matrix, as well as bone resorption [5]. The release of pro-inflammatory chemokines and cytokines, such as interleukin (IL)-6, IL-1 β , IL-8, and tumor necrosis factor (TNF)- α , is strongly associated with disease progression due to its ability to generate the resorption of the bone [6].

A higher expression of pro-inflammatory cytokines can influence the T helper cell response, producing different patterns of cytokines expression through cell subsets. A Th1 response leads to the expression of IL-2, interferon gamma (IFN- γ), and IL-12, which in turn promotes an immune cell response mostly moderated by natural killer cells, cytotoxic T cells, neutrophils, and macrophages. The Th2 responses include the expression of IL-4, IL-5, IL-6, IL-10, and IL-13, and stimulation of primarily humoral cell reaction because of antibodies produced [7].

Th17 cells are characterized by the production of IL-17 cells that are characterized by the production of IL-17 that mediates several biological functions associated with the pro-inflammatory response, including neutrophil and macrophage recruitment, stimulation of pro-inflammatory cytokine synthesis, and production of antimicrobial peptides from a variety of immune and non-immune cells [8]. Moreover, immune responses mediated by Th17 cells are highly dependent on the pro-inflammatory cytokine IL-23 that is produced by dendritic cells and is often referred to as the IL-23/Th17 axis [9].

The immune system is an equalized system and is regulated by the activity of pro/anti-inflammatory mediators and cytokines. Inflammation mediators, such as prostaglandins, cytokines, and free radicals, play a strong role in pathophysiology of disease by direct or indirect way.

There is thus an urgent need to understand peri-implant conditions to design appropriate preventive and treatment strategies.

Antioxidant and anti-inflammatory activities have been reported in some drugs of plant origin [10]. It has been observed that *Calendula officinalis*, *Salvia fruticosa*, *Achillea millefolium*, and Linn (family *Compositae*) has medical-useful features such as anti-fungal, anti-bacterial, and anti-viral activity [11]. Propolis, a natural product derived from plant resins and collected by honeybees, has been used in folk medicine throughout the world; it possesses several biological properties such as anti-inflammatory, anti-cancer, antioxidant, antibiotic, and anti-fungal activities [12]. *S. fruticosa* has been the subject of intensive study in the past decades for its antioxidative and anti-inflammatory effects in relation to active constituents, including phenolics and flavonoids [13]. The leaves and flowers of *A. millefolium* (an herb) have been used for centuries for anti-inflammatory actions [14]. Despite the traditional use of these plants, there is no scientific record of the anti-inflammatory activity of the plants *C. officinalis*, *S. fruticosa*, *A. millefolium*, or propolis as used in a single preparation.

The aim of the present study was to analyze the effect of polyphenols and flavonoids substrat (PFS) from plants *C. officinalis*, *S. fruticosa*, and *A. millefolium* as immunomodulatory in the production of IL-1 β and IL-10 in PBLM when peri-implant mucositis is diagnosed, with the intent to find new conservative methods of treatment. It was thus hypothesized that IL-1 β and IL-10 are contributing to the inflammation processes observed in the diseases of peri-implant tissues.

Material and Methods

Sampling

The study took place at the Lithuanian University of Health Sciences. Sixty patients were involved; the age limit was 55 to 70 years, and both genders were included in equal numbers. The protocol was approved by the Bioethics Committee in Kaunas (No. BE-2-76), based on Declaration of Helsinki. The patients who were included in the study signed informed consent forms.

The patients were non-smoking, were all edentulous and presented with at least 1 completely edentulous dental arch, with the intent to restore their dentition with implant-supported complete dentures. All implants were in place for at least 6 months. The mean time of implants in place was 26.3 ± 3.9 months. Each participant received 2 smooth titanium implants. Conical mini abutments measuring 3 or 4 mm in height were placed and submitted to immediate load.

The patients were divided into 2 groups: patients with healthy implants (HP group), and patients diagnosed with peri-implant mucositis affecting implants (MP group). Peri-implant

mucositis diagnosis was based on the Consensus Report of the VII European Workshop in Periodontology [15]. The implants with peri-implant gingival redness, swelling, bleeding on probing, and without radiographic signs of bone loss were considered to present as peri-implant mucositis. Patients were selected using a previous study methodology for inclusion and exclusion criteria [16]. The intraoral examination around implants was done at 6 points and evaluated bleeding, plaque, suppuration, and probing depth [16]. In addition, periapical radiographs were done [17] for patients who were diagnosed with peri-implant mucositis. For patients with healthy tissues around implants, probing was done at 6 points of each implant (in mesio-buccal and mesio-lingual, buccal, lingual, and in disto-buccal and disto-lingual) with Stoma Storz am Mark periodontal probe (Germany). Probing was done in order to evaluate these features: 1) the bleeding (presence or absence using score 1 or 0); 2) plaque evaluation being present or not using points 1 or 0); 3) suppuration, and 4) probing depth. Intraoral periapical radiographic method was used to evaluate the bone condition around each implant. All examinations of the patients were done by the same examiner, who was well trained and qualified.

Leukocytes excretion and culture

Leukocytes excretion and culture from peripheral blood was done according to Timm et al. [18]. The blood was taken in the morning and compared to the control group within 30 minutes. The peripheral blood was collected, centrifuged; then stimulated and unstimulated leukocytes were used in the study. Cells were counted using a hematological blood analyzer Sysmex xe-5000 (Sysmex Corporation, Japan). Bacteria viable *Porphyromonas gingivalis* ATCC 33277 was used for stimulation *in vitro* study (Microbiologics, Grenoble, France) [19].

Bacterial strain and culture

P. gingivalis HG91 (also designated as strain 381) was cultured anaerobically in an aerostat (Bugbox, USA) until log-growth phase in brain-heart infusion broth supplemented with hemin (5 mg/L) and menadione (1 mg/L). Purity was checked with gram-staining.

Viable *P. gingivalis* were harvested by centrifugation. Bacterial pellets were washed twice in sterile phosphate-buffered salt solution (PBS; Gibco BRL, Paisley, UK) and resuspended in antibiotic/antimycotic-free DMEM with 10% fetal bovine serum (FBS). The optical density was measured at 690 nm to establish the number of colony forming units (CFUs). A suspension of 2×10^8 CFU/mL was used to challenge the leukocytes.

Plants solution

Collaborating with a pharmacologist at Lithuanian University of Health Sciences, the plant solutions were made. They consisted of propolis, *C. officinalis*, *S. fruticosa*, and *A. millefolium* in 2 different concentrations: 5.0 mg/mL and 10.0 mg/mL.

The protocol of the experiment

The experiments were performed with unstimulated and stimulated leukocytes from the patients with healthy implants and patients with peri-implant mucositis. All the experimental techniques have been described in previous research by Akramas et al. [20]. All the study protocols were approved by our Bioethics Committee. Two systems were prepared, and 3 different samples used for each system. Both systems were then placed into a thermostat-controlled environment and maintained for 5 hours at 37°C. Then, the levels of IL-1 β and IL-10 were measured in the PBLM. The experiments with leukocytes from each patient, unstimulated and stimulated by viable *P. gingivalis*, were performed in quadruplicate.

The reagents

Hank's balanced salt solution were from Sigma Chemical Co. (St. Louis, MO, USA) and plastic vials were from Carl Rot GmbH & Co. KG (Karlsruhe, Germany) [21].

Determination of leukocytes cytokine release

All the steps were performed according to the manufacturer's recommended protocol [21]. The level of IL-1 β were investigated by standard immuno-enzymetric assay using Diasource IL-1 β ELISA kits (Belgium) and the level of IL-1 β μ g/mL was estimated. IL-10 level in the samples of PBLM were determined by the enzyme amplified sensitivity immunoassay (ELISA) method and the standard curve of optical density (OD) versus IL-10 concentration (pg/mL) was computed.

The experiments with leukocytes for each patient were performed as quadruplicates.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Software, Armonk, NY, USA). After testing for normality, parametric and nonparametric methods, the Student's *t* test, Mann-Whitney U test, and Friedman's nonparametric analysis of variance were used to compare quantitative samples. For multiple comparisons after Friedman's analysis, the Student-Newman-Keuls test used. The chi-square test was used in order to test hypotheses about independence. Discriminant analysis was applied for efficiency

Table 1. Clinical data of 2 patient groups: Healthy implants (HP) group and peri-implant mucositis (MP) group.

	HP (n=30)	MP (n=30)	P
Age (years, mean ±SD)	63.0±1.0	63.1±1.1	>0.05
Probing depth (mm, mean ±SD)	1.5±0.45	3.0±0.44	<0.001
Plaque index (mean ±SD)	0	0.89±0.10	<0.001
Marginal bleeding (mean ±SD)	0	0.79±0.11	<0.001

SD – standard deviation.

of the classification depending on the exact features. The values of threshold features were determined using retrieved linear discriminant functions which are the most effective in forecasting the HP group and the MP groups. The level of statistical significance was 0.05.

Results

The amount of IL-1β and IL-10 α cytokine production was established in a leukocyte medium produced from peripheral venous blood of patients with untreated peri-implant mucositis and after the immunomodulating effect of substrat from plants *C. officinalis*, *S. fruticosa*, *A. millefolium*, and propolis in different concentrations, and in participants with healthy implants. Table 1 shows the means and standard deviations of the groups. The average of the age of the patients having peri-mucositis and participants with healthy implants tissue was 63.1±1.1 years and 63.0±1.0 years, respectively; and there was not significant difference in age between the 2 groups ($P>0.05$). In the MP group, probing depth was bigger comparing to the HP group: 3.0 mm and 1.5 mm respectively, ($P<0.001$). Comparing the plaque index and marginal bleeding, there was noted differences in these groups, with a higher level of these features in the MP group comparing to the HP group ($P<0.001$)

Table 3. Pairwise comparisons with a Bonferroni correction, HP, IL-10.

(I) IL10	(J) IL-10	Mean difference (I-J)	P*
IL-10 uc	IL-10 u5	-0.0001	1.00
	IL-10 u10	-0.0003	1.00
	IL-10 sc	-0.0018	0.63
	IL-10 s5	-0.0031	0.00
IL-10 u5	IL-10 s10	-0.0032	0.00
	IL-10 u10	-0.0002	1.00
	IL-10 sc	-0.0017	0.64
	IL-10 s5	-0.0030	0.01
IL-10 u10	IL-10 s10	-0.0031	0.02
	IL-10 sc	-0.0016	1.00
	IL-10 s5	-0.0028	0.01
	IL-10 s10	-0.0029	0.00
IL-10 sc	IL-10 s5	-0.0013	0.93
	IL-10 s10	-0.0014	1.00
IL-10 s5	IL-10 s10	-0.0001	1.00

* Adjustment for multiple comparisons: Bonferroni. HP – healthy patients; uc – unstimulated PBLM and control; u5 – unstimulated PBLM and 5.0 mg/mL PSF; u10 – unstimulated PBLM and 10.0 mg/mL PSF; sc – stimulated PBLM and control; s5 – stimulated PBLM and 5.0 mg/mL PSF; s10 – stimulated PBLM and 10.0 mg/mL PSF.

Suppuration was not fixed in any group. No significant differences were noticed between the 2 groups when evaluating their mean leukocyte count in the incubation medium ($P>0.1$), Table 2.

A dispersal analysis of repeated measurements was performed for the comparison of IL level averages at different measuring conditions. The within-subjects factor had 6 measurements:

Table 2. Leukocyte count and percentage distribution in peripheral blood incubation medium.

Groups	Leukocyte count (LC) in incubation medium (cells 1×10 ⁹ /L)			
	Differential LC	Granulocytes %	Lymphocytes %	Monocytes %
HP group (n=30)	10.1±0.12	43.8±6.4	54.5±7.8	2.8±1.2
MP group (n=30)	10.3±0.14	45.5±7.5	52.9±8.2	2.8±1.5
P	$P>0.1$	$P>0.1$	$P>0.1$	$P>0.1$

HP – patients with healthy implants; MP – patients with untreated peri-implant mucositis.

Table 4. Pairwise comparisons with a Bonferroni correction, MP, IL-10.

(I) IL-10	(J) 1 β	Mean difference (I-J)	P*
IL-10 uc	IL-10 u5	-0.001	1.000
	IL-10 u10	-.013	0.000
	IL-10 sc	-.008*	0.000
	IL-10 s5	-.017	0.000
	IL-10 s10	-.043	0.000
IL-10 u5	IL-10 u10	-.012	0.000
	IL-10 sc	-.008	0.000
	IL-10 s5	-.016	0.000
	IL-10 s10	-.042	0.000
IL-10 u10	IL-10 sc	.004	0.000
	IL-10 s5	-0.004	0.115
	IL-10 s10	-.030	0.000
IL-10 sc	IL-10 s5	-.008	0.000
	IL-10 s10	-.035	0.000
IL-10 s5	IL-10 s10	-.027	0.000

*. Adjustment for multiple comparisons: Bonferroni.
MP – patients with peri-implant mucositis; IL – interleukin;
uc – unstimulated PBLM and control; u5 – unstimulated PBLM and 5.0 mg/mL PSF; u10 – unstimulated PBLM and 10.0 mg/mL PSF; sc – stimulated PBLM and control; s5 – stimulated PBLM and 5.0 mg/mL PSF; s10 – stimulated PBLM and 10.0 mg/mL PSF.

1) unstimulated PBLM and control (uc); 2) unstimulated PBLM and 5.0 mg/mL PSF (u5); 3) unstimulated PBLM and 10.0 mg/mL PSF (u10); 4) stimulated PBLM and control (sc); 5) stimulated PBLM and 5.0 mg/mL PSF (s5); and 6) stimulated PBLM and 10.0 mg/mL PSF (s10). Dispersion analysis of repeated measurements was performed separately for the HP group and the MP group.

IL-10 and IL-1 β

In the HP group, the IL-10, the within-subject factor effects had a statistically significant influence ($F=7.58, P<0.01$). Pairwise comparisons are presented in Table 3.

In the MP group, the IL-10, the within-subjects factor effect also had a statistically significant influence ($F=421.18, P<0.01$). Pairwise comparisons are presented in Table 4.

Table 5. Pairwise comparisons with a Bonferroni correction MP, IL-1 β .

(I) IL-1 β	(J) 1 β	Mean difference (I-J)	P*
IL-1 β uc	IL-1 β u5	.004	0.00
	IL-1 β u10	.006	0.00
	IL-1 β sc	-.006	0.00
	IL-1 β s5	0.0006	1.00
	IL-1 β s10	.013	0.00
IL-1 β u5	IL-1 β u10	.003	0.02
	IL-1 β sc	-.009	0.00
	IL-1 β s5	-0.0030	0.24
	IL-1 β s10	.010	0.00
IL-1 β u10	IL-1 β sc	-.012	0.00
	IL-1 β s5	-.006	0.00
	IL-1 β s10	.007	0.00
IL-1 β sc	IL-1 β s5	.006	0.00
	IL-1 β s10	.019	0.00
IL-1 β s5	IL-1 β s10	.013	0.00

*. Adjustment for multiple comparisons: Bonferroni.
uc – unstimulated PBLM and control; u5 – unstimulated PBLM and 5.0 mg/mL PSF; u10 – unstimulated PBLM and 10.0 mg/mL PSF; sc – stimulated PBLM and control; s5 – stimulated PBLM and 5.0 mg/mL PSF; s10 – stimulated PBLM and 10.0 mg/mL PSF.

In the MP group, the IL-1 β within-subjects factor effects had a statistically significant influence ($F=61.68, P<0.01$). Pairwise comparisons are presented in Table 5.

In the HP group, the IL-1 β within-subjects factor effects was not statistically significant ($F=3.31, P>0.05$). There was no statistically significant difference between the pairwise comparisons ($P>0.05$).

The results for the analyzed variables of IL-1 β and IL-10 levels in the 2 systems of venous blood leukocytes assays of the MP group and the HP group following the effects of PFS with different concentrations are given in Figures 1 and 2.

In Figure 1, the estimate marginal means of IL-10 levels in PBLM (significant differences at IL-10 was u10, IL-10 was sc, IL-10 was s5 and IL-10 was s10, measuring occasion). In Figure 2 the estimate marginal means of IL-1 β levels in PBLM (significant difference for IL-1 β was u5, IL-1 β was u10, IL-1 β was sc, and IL-1 β was s10, measuring occasion).

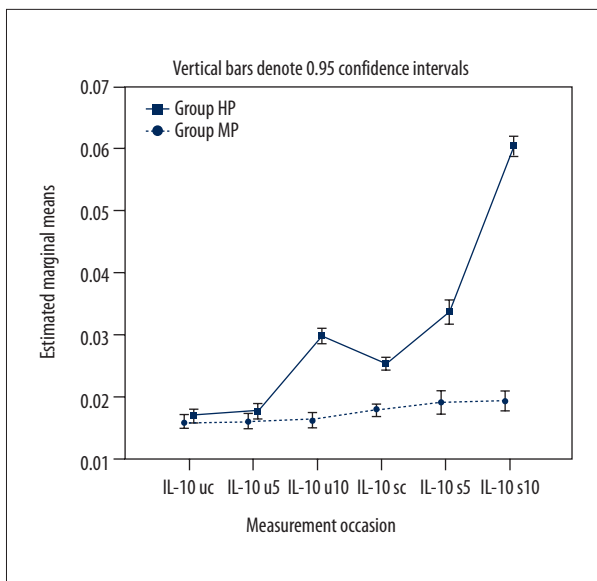


Figure 1. IL-10 levels in unstimulated and stimulated by viable *Porphyromonas gingivalis* leukocytes medium and treated with PFS. Significant differences at IL-10 u10, IL-10 sc, IL-10 s5 and IL-10 s10 measuring occasion. Remaining results indicate a statistically significant difference $P < 0.001$. IL-10 – interleukin-10 (pg/mL); PFS – the polyphenols and flavonoids substrat; uc – unstimulated PBLM and control; u5 – unstimulated PBLM and 5.0 mg/mL PSF; u10 – unstimulated PBLM and 10.0 mg/mL PSF; sc – stimulated PBLM and control; s5 – stimulated PBLM and 5.0 mg/mL PSF; s10 – stimulated PBLM and 10.0 mg/mL PSF.

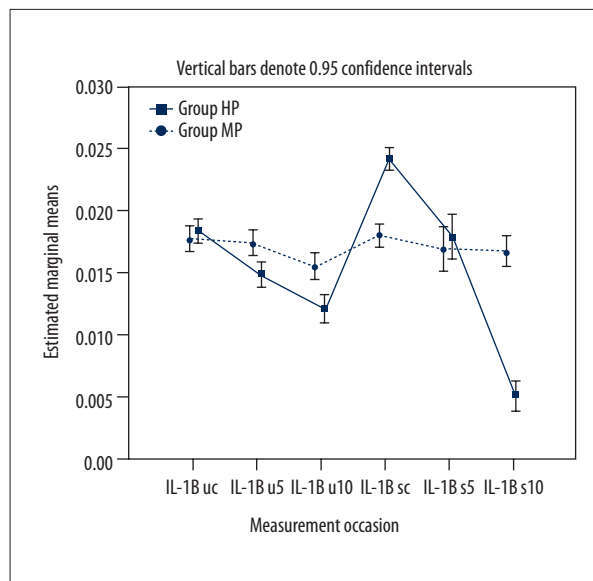


Figure 2. IL-1β levels in unstimulated and stimulated by viable *Porphyromonas gingivalis* leukocytes medium and treated with PFS. Significant differences at IL-1β u5, IL-1β u10, IL-1β sc and IB-1β s10 measuring occasion. Results indicate a statistically significant difference $P < 0.001$. IL-1β – interleukin-1β (pg/mL); PFS – the polyphenols and flavonoids substrat; uc – unstimulated PBLM and control; u5 – unstimulated PBLM and 5.0 mg/mL PSF; u10 – unstimulated PBLM and 10.0 mg/mL PSF; sc – stimulated PBLM and control; s5 – stimulated PBLM and 5.0 mg/mL PSF; s10 – stimulated PBLM and 10.0 mg/mL PSF.

Discussion

Because peri-implant mucositis is the first sign which if untreated leads to peri-implantitis, early diagnosis and effective treatment of mucositis is an appropriate way to prevent peri-implantitis [22]. It can establish some changes in affected area, such as increased blood flow and vascular permeability, and also exudation of fluid and leukocytes activity such as migration and accumulation which shows inflammatory process [23].

Immunoglobulin production can be caused by T-helper 1 (Th1) immune response via Th2 response, while IL-10 is an anti-inflammatory cytokine and it can redirect T-helper immune reaction [24,25]. It has been established that there is a difference in IL-10 in patients with mucositis diagnosis compared to patients with peri-implantitis, and accordingly lower numbers with mucositis and higher numbers with peri-implantitis [26]. These higher levels of IL-10 in stimulated PBLM with mucositis of peri-implant tissues might be explained by the increased amount of cytokines in peri-implant tissues compared to their amount in the peri-implant fluid. It was thought that IL-10 inhibits cytokine synthesis, but recent data suggests it has an immune-regulatory role. L-10 is also known for its capability to

inhibit differentiation of monocyte to antigen-presenting cells, and also repress the expression of most inducible chemokines, which takes place in the inflammatory processes. It is known that IL-10 inhibits bone resorption by suppressing cyclooxygenase-2-dependent prostaglandin E2 synthesis, and enhances the production of anti-inflammatory mediators; other stimulatory effects of L-10 contain B cell processes of proliferation and differentiation and helps them develop into plasma cells. IL-10 also inhibits recruitment of osteoclast precursors and their differentiation to mature multinucleated osteoclasts [27].

Pro-inflammatory cytokines (TNF-α, IL-1β) are known as important factors of inflammatory host reaction to infection and other inflammatory irritants. In order to determine the severity of inflammation of peri-implant tissues, appropriate indicator can be IL-1β as it is a pro-inflammatory cytokine which regulates the degradation of the connective tissue and modulates the reparative activity indicating the endothelial cells to cause proliferation of fibroblasts and chemotaxis of neutrophils in inflamed gingiva. The levels of IL-1β can help measure the severity of inflammatory periodontal disease [21]. The concentrations of IL-1β in peri-implant crevicular fluid (PICF) are seen

to be at a higher level even in the very first stages of peri-implantitis; the inclination for it later to increase is seen related to the progression of the disease [28].

TNF- α and IL-1 β act synergically, including stimulation of bone resorption. These 2 primary cytokines can also cause the occurrence of adhesion molecules and secondary mediators, and these processes enlarge the inflammatory response, causing matrix metalloproteinase (MMP) production, and bone resorption [29].

Recent studies have demonstrated the role of pro-inflammatory cytokines TNF- α and IL-1 β in peri-implantitis. It has been noted that they have a strong impact in the destruction processes by inducing and increasing bone resorption, and they cause the release of MMP resulting in degradation of proteins of extracellular matrix and also affect the systemic manifestation of inflammation [30,31]. The comprehension of immune reaction to inflammation processes at the cellular level allows searching the specific working mechanism to control it at the beginning. Nowadays development of medicine is concerned with more natural treatments, that is why it is important to find the pharmaceutical effect of any plants. For example, *A. millefolium* L.s.1. is traditionally used not only in the treatment of gastrointestinal conditions, but also as considered an antiphlogistic drug; while a topical anti-inflammatory activity of the sesquiterpenes has been shown to be caused by inhibition of arachidonic acid metabolism [32,33].

In vitro and *in vivo* studies have shown that the medical impact of *C. officinalis* Linn flower extract possesses many radicals, suppresses lipid peroxidation and enhancing antioxidant status [34]. Also, this extract has anti-inflammatory activity, affecting Cox-2, IL-1 β , IL-6, TNF- α , IFN- γ , acute phase protein, and C reactive protein. To sum up, an anti-inflammatory response of *C. officinalis* extract might be explained by the inhibition of pro-inflammatory cytokines and Cox-2 and subsequent prostaglandin synthesis [34].

The species *S. fruticosa* Miller, is widely used in folk medicine. Accordingly, the present study was designed to evaluate the antioxidant and anti-inflammatory activities of *S. fruticosa*, and to determine the phenolic constituents of its extracts. *S. fruticosa* was found to be a rich source of antioxidants, where the ethyl acetate root extract had the highest radical scavenging activity and the highest concentration of phenolics. The aerial parts of the plant showed the higher anti-inflammatory activity. Both the aerial parts and the roots exhibited significant protection against carrageenan-induced mouse paw edema with promising activities of the 2 crude extracts relative to that of the standard drugs diclofenac reaching 90%. The anti-inflammatory activity of the extracts was in line with their antioxidant activity better than their phenolic contents. The observed

anti-inflammatory activities of this extract might partly be attributed to the overall effects of the phenolics and other plant constituents having potent anti-inflammatory actions similar to diclofenac [35].

Propolis, a natural product derived from plant resins and collected by honeybees, has been used in folk medicine. It possesses several biological properties such as anti-inflammatory, anticancer, antioxidant, antibiotic and antifungal activities. Borrelli et al. reported that caffeic acid phenethyl ester (CAPE) is a primary component of propolis, and inhibits carrageenan hind paw edema, carrageenan pleurisy, and adjuvant arthritis in rats, suggesting that the anti-inflammatory activity of propolis is due to CAPE. Moreover, in *in vitro* experiments, CAPE inhibits the production of nitric oxide and the activation of nuclear transcription factor NF-Kb, which are associated with inflammation [36].

Our findings confirmed the data of other studies [33–36], which indicated that the solution of the plants made up of *C. officinalis*, *S. fruticosa*, *A. millefolium*, and propolis, had a helpful effect on controlling the inflammation process. There has also been some research regarding phyto-drugs in dentistry. For example, the phyto-therapeutic drug composed of herbal extract (i.e., baicalin, bromelain, and escin) showed a decrease in the severity of postoperative pain after surgical removal of impacted mandibular third molar compared to ibuprofen and placebo [37]. An *in vitro* study using different concentration ranges of plants solution evaluated if the compound had an incentive effect on leukocytes. Genetic polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) enzyme can influence alterations in DNA methylation and it has been shown that hypermethylation of cancer-related genes might be affected by MTHFR polymorphisms [38]. The transforming growth factor-B1 (TGF- β 1) and vascular endothelial growth factor (VEGF) are also thought to play an important role in proliferation and/or migration of structural cells involved in inflammation and regulation of immune response, which in turn might influence the outcome of disease establishment and evolution [39]. We found that PBLMs unstimulated and stimulated by viable *P. gingivalis* and treated with 5.0 mg/mL or 10.0 mg/mL of PFS in the MP group produced significantly higher levels of IL-10 ($P < 0.001$, $P < 0.001$, respectively) than the analogous PBLMs of HP group. After treatment with PFS, only with concentrations of 10.0 mg/mL did the IL-1 β levels decrease more considerably in the stimulated PBLMs of the MP group than in the HP group ($P < 0.001$). Our findings indicated that PFS can affect the humoral immunity in positive way and also affect the cellular immunity by reducing the pro-inflammatory cytokines, thus boosting the increase of anti-inflammatory cytokines and controlling inflammation progression.

Conclusions

Our results suggested that the solution of the plants that includes *C. officinalis*, *S. fruticosa*, *A. millefolium*, and propolis could be under consideration as a new therapeutic method to prevent and treat inflammatory processes of peri-implant tissues even in early stages.

Limitations of study

There were several limitations to this study. There was a small number of cases in both subgroups, and that might have an impact on our findings. A larger study is necessary to explore the significance of cytokine IL-10 and IL-1 β in the progression of chronic peri-mucositis.

Conflict of interests

None.

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