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Effect of 2% quercetin gel subgingival application after scaling and root planing on IL-6 concentration of chronic periodontitis patients

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Abstract
Quercetin is an anti-inflammatory substance that possesses strong anti-inflammatory capacities through reduction of proinflammatory cytokines. Interleukin-6 is a proinflammatory cytokine produced locally in inflammatory tissue after lipopolysaccharides and other cytokines activation. The research analyzed effect of 2% quercetin gel subgingival application after scaling and root planing on IL-6 concentration of chronic periodontitis patients. It used 2% quercetin gel after scaling and root planing and 0,2% chlorhexidine gel as control. Sample was divided into 3 groups, quercetin gel group, control group (chlorhexidine gel and without gel). Gingival crevicular fluid was taken before and after treatments in each group then IL-6 examination was performed. This research used pretest-posttest control group design. There was significant difference (p<0,05) on the IL-6 concentration reduction before and after 2% quercetin gel application. Quercetin gel application reduced IL-6 concentration in gingival crevicular fluid of periodontitis patients.

Keywords: Quercetin, IL-6, periodontitis, cytokines, osteoclasts

1. Introduction
Quercetin is a group of flavonols found in vegetables, fruits and nuts such as shallots, cranberries, blueberries, tomatoes, broccoli, and apples. It also provides a variety of health benefits and resistance to diseases including anti carcinogenic, anti-inflammatory, antiviral, antioxidant, psychostimulant activity and also ability in inhibiting lipid peroxidation, platelet aggregation, capillary permeability and stimulation of mitochondrial biogenesis. Quercetin is reported as anti-inflammatory substance that last long and have strong anti-inflammatory capacities. It has demonstrated antimicrobial activity that potentially decrease the inflammatory marker, cholesterol reduction and inhibit bone loss [1-4].
Periodontitis is a chronic inflammatory disease affecting the dental supporting tissue [5-6]. It is characterized by changes in the color, texture and volume of gingival margin, bleeding on probing, periodontal pocket, loss of attachment level, gingival recession, alveolar bone loss, molar furcation exposure, mobility, drifting and tooth loss [1, 7-8]. The etiology of periodontitis is multifactorial which involves bacteria, genetic factors, environment and host [1, 5-6]. Pathogenesis of periodontitis can be broadly divided into 2 parts namely derived from subgingival bacteria and inflammatory-immune host response but more tissue destructions are caused by inflammatory processes. Subgingival bacteria also contribute directly to the breakdown of periodontal tissue by removing the harmful substance but the most important bacterial role in the pathogenesis of periodontitis is to activate the inflammation-immune response of the host, resulting in damage to periodontal tissue. Cytokines are produced by cells in nonspecific immunity are the earliest defensive lines against pathogens. These cells have receptors that recognize bacterial patterns thus allowing a response to bacterial invasion by producing cytokines that activate B cells and T cells in specific immunity. Excessive cytokine production will trigger tissue damage which depicting clinical signs of periodontal disease [5, 7].
Interleukin-6 (IL-6) is found in cells, tissues and gingival crevicular fluid patients who suffer periodontal disease. Its secretion is stimulated by Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α) and are produced by immune cells such as B cells, T cells, macrophages,

dendritic cells, keratinocytes, endothelial and fibroblasts. IL-6 is also secreted by osteoblasts and stimulates the occurrence of bone resorption and the development of osteoclasts [7, 8]. Research conducted by Zeynep *et al.*, against 30 patients with periodontal disease showed that IL-6 in gingival and serum creatinine fluid significantly [9].

This research aimed to analyze the effect of quercetin gel subgingival application after scaling and root planing on IL-6 reduction and analyzed the correlation of IL-6 concentration reduction with clinical parameters of chronic periodontitis patients.

2. Materials and methods

The population of this study were patients with chronic periodontitis who came for treatment at USU RSGMP Periodontia Installation. The sample was gingival crevicular fluid taken from chronic periodontitis patients who were treated at the USU RSGMP Periodontia Installation. Determination of the research sample was done by purposive sampling technique. Ethical clearance approval was obtained from the ethics commission and study participants were given an explanation of the procedure from the beginning to the end of the study and signed an informed consent.

Inclusion criteria was patients with chronic periodontitis (35-60 years old) with pockets depth (PD) 4-5 mm, number of teeth in the oral cavity at least 15 teeth, willing to undergo examination (followed the research procedure and signed informed consent) while patients who used mouthwash regularly, smoking, suffer from systemic diseases, had periodontal treatment in the last 6 months, pregnant women and breastfeeding mothers, consuming alcohol and drugs such as immunosuppressant, calcium channel blockers, cyclosporine, antibiotic and anti-inflammatory were excluded from the study.

Samples of research divided into three groups, quercetin gel group (scaling and root planing combined with 2% quercetin gel), the control groups were group of chlorhexidine gel (scaling and root planing combined with 0,2% chlorhexidine gel) and group without gel administration (scaling and root planing).

Clinical examinations were conducted on 1st and 7th day with clinical examinations of the Gingival Index (GI) and Papillary Bleeding Index (PBI). The basic components of the gel were carbopol (1 gr), HPMC (1 gr), TEA (3 gr), glycerin (4 gr), nipagin (0.04 gr), nipasol (0.04 gr) and aquadest [10].

A sample of gingival crevicular fluid was taken using micropipette (1-10 µl) before and after application of quercetin gel. The tip of the micropipette was inserted slowly into a pocket of gingiva and should not be contaminated with blood and plaque. The sample was inserted into an eppendorf tube measuring 10 µl, labeled and stored in a cooling box. Quercetin gel was applied topically into periodontal pocket area after scaling and root planing treatment. The applied area was dried using a wind spray and isolated by a cotton roll. After the gel applied to the specified area and covered with periodontal pack (Coe-pack ™). Patients were instructed not to eat for about an hour after administration of the gel and to keep the oral hygiene. The patients were asked to come back on the 7th day to collect gingival crevicular fluid with the same procedures on the 1st day. The laboratory procedure was carried out using the enzyme-linked immunosorbent assay (ELISA) method. The difference of IL-6 concentration before and after scaling and root planing of quercetin gel group was analyzed using a statistical test of Wilcoxon.

3. Results and discussion

The difference of IL-6 concentration and clinical parameters were measured to determine the effectiveness of using the gel as an adjunctive was proven to reduce the inflammation. It's shown on Tables 1, 2, 3.

Table 1: The difference of IL-6 concentration on 1st and 7th day of each group

Group	1st Day	7th Day	Average decrease	*p* value
	Mean ± SD (pg/ml)	Mean ± SD (pg/ml)	Mean ± SD (%)	
Quercetin Gel[a]	160,40±23,10	92,40±29,02	43,49±11,28	0,028*
Chlorhexidine Gel[b]	179,00±40,68	146,81±35,11	18,12±1,75	0,000*
Without Gel[b]	193,80±35,17	178,40±32,99	7,97±1,94	0,000*

[a]Wilcoxon test
[b]Paired t test
(*) Significant $p<0.05$

Quercetin gel group on IL-6 concentration had the largest average decrease among the groups. The difference of IL-6 concentration of quercetin gel had a significant difference ($p<0,05$) (Table 1).

Table 2: The difference of Gingival Index on 1st and 7th day of each group

Group	1st day Mean ± SD	7th day Mean ± SD	Average decrease Mean ± SD (%)	*p* value
Quercetin Gel[a]	1,18±0,24	0,13±0,04	89,24±1,41	0,028*
Chlorhexidine Gel[b]	1,20±0,33	0,53±0,15	56,03±3,43	0,000*
Without Gel[b]	1,30±0,35	0,62±0,17	52,61±3,52	0,000*

[a]Wilcoxon test
[b]Paired t test
(*) Significant $p<0.05$

Quercetin gel group on Gingival Index (GI) parameter had the largest average decrease among the groups. The difference GI of quercetin gel group had a significant difference ($p<0,05$) (Table 2).

Table 3: The difference of Papillary Bleeding Index on 1st and 7th day of each group

Group	1st day Mean ± SD	7th day Mean ± SD	Average decrease Mean ± SD	*p* value
Quercetin Gel[a]	7,17±0,75	2,83±0,41	60,51±4,00	0,023*
Chlorhexidine Gel[b]	7,00±0,89	3,67±0,52	47,22±8,17	0,000*
Without Gel[b]	7,83±0,75	4,67±0,52	40,44±3,28	0,000*

[a]Wilcoxon test
[b]Paired t test
(*)Significant $p<0.05$

Quercetin gel group on Papillary Bleeding Index (PBI) parameter had the largest average decrease among the groups. The difference PBI of quercetin gel group had a significant difference ($p<0,05$) (Table 3).

Table 4: The correlation of IL-6 concentration of clinical parameters GI and PBI (N = 18)

Parameters	GI	PBI
IL-6	0,899**	0,802**

Pearson correlation test
(**) Significant Correlation

There was a positive, strong and significant correlation between IL-6 concentration correlated with clinical parameters (Gingival Index and Papillary Bleeding Index) (Table 4).

In this study, there was statistically significant decrease of IL-6 concentrations after 7th day (Table 1) and the quercetin group found a greater percentage reduction due to quercetin having the ability to inhibit NF-κB activity by increasing the complex members of the IKK so that the NF-κB complex would not be split and activated [11]. One of the most dependent cytokines in NF-κB induction is IL-6 [12]. IL-6 is as same as other cytokines requiring genetic regulation before being secreted, it relies on the activation of NF-κB. NF-κB is activated by molecular patterns related to pathogens such as LPS. After the activation, NF-κB will directly transcribe the cytokine gene in the nucleus in particular IL-6 so that the cell will secrete the IL-6 [13, 14]. Takashima *et al*. revealed that quercetin decreased the production of TNF-α, IL-1β and IL-6 through research carried out against LPS-induced bronchoalveolar. May and Gosh said that activated TLRs triggers NF-κB stimulation there by reducing the activity of major inflammatory mediators [15].

Clinically significant decrease was found in statistical parameters after 7th day in the whole groups (Table 2 and Table 3). Gingival inflammation is marked by erythema and oedema on gingival. It's caused by biofilm formation, pathogenic microbiota on subgingival area which trigger immune response leades to tooth supporting tissue damage. [16] Clinical parameters decrease by quercetin gel due to its ability to inhibit the activation of NF-κB and to induce molecules that function extensively in recruiting leukocytes including vascular adhesions resulting in improvement of clinical parameters [17].

In this study there was strong and positive correlation between the IL-6 and the clinical parameters of GI and PBI, this is in line with the research conducted by Abdulkareem *et al*. in 101 patients who gained a correlation of IL-6 with bleeding on probing (BOP) of 0.664 in chronic periodontitular patients [18]. Abdulkareem H *et al.* also gained a significant correlation of moderate positive between the BOP and the IL-6 level in, (0608) P-value (0.001). The cytokines increase the recruitment of neutrophil cells and the expression of the molecular adhesion that causes vascular modification [19] Honda *et al.* said the intensity, duration, and inflammatory resolution depend on balancing of pro and anti-inflammatory cytokines activity during periodontal inflammatory tissue occurred [18]

4. Conclusion

The study concluded that quercetin gel application reduced IL-6 concentration in gingival crevicular fluid of periodontitis patients.

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