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Evaluation of il-6 levels after subgingival application Binahong leaf extract gel 3% on patients of chronic periodontitis

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Abstract
Chronic periodontitis is an inflammatory disease which is characterized by the absence of pain and develops slowly, loss of attachment and resorption of bones that causes teeth to mobile and loose. Some markers responsible for the inflammatory process include cytokines, such as Interleukin-6 (IL-6), as one of the inflammatory cytokines, plays an important role in the inflammatory response of the periodontium. To handle the case of periodontitis, can be done by scaling and root planning (SRP) and can be combined with systemic ingredients and the application of herbal ingredients. One of the natural ingredients that has been used in wound healing is binahong leaves. In this study using 3% binahong leaves gel extract was applied to subgingival to reduce levels of IL-6 and periodontal status. In this study, using 16 samples, were divided into 2 groups: ie 8 samples as a test group, and 8 samples as a control group. IL-6 levels were examined after scaling and root smoothing. Statistically, there was a significant decrease in IL-6 levels in the gingival sulcus fluid after scaling and root planing (SRP) in periodontitis patients with ap values <0.05. There was a significant decrease in measurement of IL-6 levels in the test and control groups after application of subgingival gel of binahong leaves extract (*Andredera cordifolia* (Ten.) Steenis) 3% in patients with chronic periodontitis. there was a significant decrease in IL-6 levels in the gingival sulcus fluid after scaling and root planing (SRP) in periodontitis patients with ap value <0.05.

Keywords: Binahong, IL-6, chronic periodontitis

Introduction
Interim prostheses are essential components of fixed prosthodontic treatment. These restorations should fulfill biological, mechanical and esthetic requirements to be considered successful [1]. The interim restorative materials should possess a number of ideal mechanical and physical properties, such as a high flexural strength, increased resistance to wear, high fracture strength, dimensional stability, minimal marginal gap formation and increased resistance to staining and discoloration [2]. One of the important aspects of provisional restorations is their flexural strength which plays a critical role in both functional as well as parafunctional conditions. Inadequate flexural strength may cause difficulty for both the patient and clinicians to keep the interim restorations intact [3]. The elastic modulus or modulus of elasticity is also an important factor in interim restorations as it describes the relative stiffness or rigidity of a material used for the fabrication of interim fixed prosthesis. Recent studies have evaluated flexural strength and elastic modulus of various materials, but very little emphasis has been laid on the impact of varying the thickness of restorations on the above two parameters. The purpose of the present *in-vitro* study is to assess the flexural strength and elastic modulus of three commonly used interim restorative materials using different thickness to cover a wider range of clinical applicability. The present *in-vitro* study was conducted in the Department of Prosthodontics, BRS Dental College & Hospital, Sultanpur, Panchkula.

Materials and Methodology
A brass die was fabricated according to American National Standards Institute/ American Dental Association specification no. 27 (dimensions 25×2×2mm) [4], with change in thickness of 0.5mm (25×2×2mm,25×2×2.5mm and 25×2×3mm) in order to simulate the oral conditions

Chronic periodontitis is an inflammatory disease characterized by the absence of pain and develops slowly, loss of attachment and resorption of bone that causes teeth to shake and date.1 Loss of attachment and resorption of bone is known to be associated with an increase in the number of gram-negative organisms in subgingival biofilm plaques. However, the mechanism that occurs is not yet explained with certainty, but bacteria can have a local effect on inflammatory response cells, as well as host cells and tissues, which produce a process of disease development that is local and specific.2

Interleukin-6 (IL-6) is a proinflammatory cytokine that can be found in acute and chronic inflammation.3 IL-6 is also able to stimulate a number of biological processes such as antibody and autoantibody production, T-cell activation, B-cell differentiation, and osteoclasts differentiation.4 IL-6 present in inflamed gingival tissue can contribute to the development and pathological activity of periodontal disease.5

Binahong (Anredera cordifolia (Ten.) Steenis) is a natural plant that has anti- inflammatory effects and can be used for the treatment of periodontitis.6,7 Binahong leaf extract has been shown to produce various compounds such as saponins, alkaloids, phenolics, and flavonoids which show some interesting effects such as anti- inflammatory, antibacterial, and antioxidant properties.8 Anredera cordifolia extract is reported to have potential anti-inflammatory properties by inhibiting inflammatory mediators including TNF-α, IL-1β, IL-6, and NO in macrophage cells induced by LPS.6 Binahong leaf extracts play a role in wound healing, where the flavanoid content is responsible for anti-inflammatory mechanisms, inhibits free radical activity, and increases epithelialization in open wounds on rabbit skin.9 The flavonoid component itself is known to be able to reduce the amount of IL-6 production, where IL-6 plays an important role in regulating the function of the immune system and is produced during an infection.10 Binahong leaves also have saponin compounds which compound is divided into several components, namely steroidal saponins, triterpenoidal saponins and alkaloid saponins depend on the structure of the aglycone, where steroids themselves in modern clinical studies have a role as anti-inflammatory and analgesic agents.11

This study aims to determine the effect of the subgingival application of binahong leaf extract (Anredera cordifolia (Ten.) Steenis) 3% on decreasing levels of gingival crevicular fluid interleukin-6 and affecting the periodontal status of patients with chronic periodontitis.

Materials and Methods

The study population was all patients with chronic periodontitis who came for treatment at the USU RSGMP Periodontia Installation. The sample was gingival

crevicular fluid taken from chronic periodontitis patients who were treated at the USU RSGMP Periodontia Installation and had fulfilled the inclusion and exclusion criteria.

Determination of the research sample is done by pusposive sampling technique. Ethical clearance approval was obtained from the ethics commission and study

participants were given an explanation of the study procedure from the beginning to the end of the study and signed an informed consent.

Inclusion criteria

1. Minimum number of teeth is 12 teeth
2. 40-60 years old
3. Patients with chronic periodontitis with a pocket depth of 5-7 mm on one side
4. Willing to undergo examination and follow the research procedures, as well sign informed consent
5. Do not smoke

Exclusion Criteria

1. Patients with severe crowded teeth, severe caries, grade III mobility teeth and roots.
2. Use mouthwash regularly and consume drugs
3. Patients who have treated their oral cavity in the past 6 months
4. Patients with systemic diseases who routinely use drugs
5. Pregnant and breastfeeding
6. Consuming alcohol

The research sample was divided into two groups, namely the treatment group (application of 3% binahong leaf extract gel) and the control group.

Research procedure

Gel making procedure

The basic components of making binahong leaf gel (Anredera cordifolia (Ten.) Steenis) 3% were obtained from Hanafiah's research, namely: Carbopol (1 gr), HPMC (1 gr), TEA (3 gr), Glycerin (4 gr), Nipagin (4 gr) 0.04 gr), Nipasol (0.04 gr) and Aquades. In this study, to get 40 grams of gel 3% was obtained from the calculation of 3 g / 100 g x 40 g = 1.2 g extract.12

Procedure for taking gingival crevicular fluid

Crevicular fluid samples were taken before and after the application of 3% binahong leaf extract gel. Crevicular fluid is taken from the molar teeth or incisor with the deepest intrasulcular pocket on the study subjects. Patients were instructed not to eat and brush their teeth ≥ 1 hour before the study. Before being taken, the tooth area is cleaned with cotton /cotton roll to control saliva. Gingival crevicular fluid was taken using microcapillary size 1-10 µL which was mounted on a micropipette. The tip of the microcapillary tip is inserted slowly into the pocket. Gingival crevicular fluid must not be contaminated with blood and plaque. Then the micropipette is pulled so that the gingival crevicular fluid is sucked into the microcapillary. The gingival crevicular fluid is then put into a 10µL eppendorf tube and stored in a cooling box, and stored in the refrigerator.

Scaling and root planning

Application procedure for binahong leaf extract gel 3% Application of topical 3% binahong leaf extract gel ingredients in the gingival pocket area that has been determined using *syringe* with a blunt needle until the gel appears out of the gingival margin and the excess gel is cleaned with a cotton swab in the treatment group sample. Avoid eating and drinking, gargling and spitting at least 1 hour after gel application. The application is carried out by the operator on day-0 (after SRP), day-3 and day-5. This is based on the length of the inflammatory process in wound healing (after SRP). Patients were asked to come back on the 7th day to collect crevicular fluid. The procedure for taking crevicular fluid on day 7 is the same as the procedure for taking gingival crevicular fluid before applying the gel.

Laboratory procedures

Examination of gingival crevicular fluid samples is carried out by the *enzyme- linked immunosorbent assay* (ELISA) method.

Statistical analysis

Data processing in this study was carried out using a computerized system that is displayed in tabular form. Data analysis in this study was carried out after examination of interleukin-6 levels in the gingival sulcus fluid in the USU FK laboratory. The normality test uses the Saphiro Wilk test to see the distribution of group data and a t-paired test (if normally distributed) to look at IL-6 levels of gingival sulcus fluid after scaling and root planning (SRP) in patients with chronic periodontitis. Furthermore, an independent t-test was performed on differences between groups to see IL-6 gingival sulcus fluid which, if normally distributed.

Results and Discussion

Results *p-value* obtained in the test and control group by 0,000 which means a significant decrease in sulcus fluid IL-6 levels between before and after treatment

(Table 1). There was a very significant change in Δ IL-6 level ($p< 0.05$) (Table 2).

Table 1: The difference in IL-6 levels test after treatment in the test and the control group.

Variable	Test		*P*	Control		*P*
	Before	After		Before	After	
	(X ± SD)	(X ± SD)		(X ± SD)	(X ± SD)	
IL-6 (pg / ml)	265.79 ± 45.20	81.19 ± 15.52	0,000 *	200.74 ± 31.81	191.19 ± 34.24	0,000 *

Paired t-test: * Significant $p< 0.05$

Table 2: Differences in Difference of Clinical Parameters and IL-6 Levels between Test and Control Groups

Clinical Parameter	Test (X ± SD)	Control (X ± SD)	*P*
Δ IL - 6	184.60 ± 38.71	9.55 ± 7.73	0,000 *

Independent t-test

* Significant $p<0.05$

Δ is: Difference before and after treatment

In this study, a significant decrease in IL-6 levels in the gingival sulcus fluid before and after SRP was performed in both the test and control groups. The several studies show results that are in line with the above research. Among them, the study of Goutoudi *et al*. which found an average IL-6 concentration of gingival sulcus fluid between the periodontitis and healthy controls after 6 weeks of scaling and root planing (SRP) had a significant decrease.15 Nanakaly, *et al* found that the average concentration of IL- 6 in saliva before and after scaling and root planing (SRP) of chronic periodontitis patients and control groups had a significant decrease.16 This shows that SRP can reduce the amount of IL-6 contained in the gingival sulcus fluid. Based on the statistical results in this study, a significant difference was obtained between the test group and the control group, namely a decrease in IL-6 values. This is supported by research on the content of binahong leaves from phytochemical tests conducted by Hanafiah OA and Anasta which show that binahong leaves contain saponin, tannin, triterpenoid, alkaloid, flavonoid, phenolic, steroid, β-sitosterol glycoside compounds which have anti-inflammatory effects.12,17,18 Yuziani tested the anti-inflammatory effect of binahong leaf extract which has saponin compounds where the compound is divided into several components, namely steroidal saponins, triterpenoidal saponins and alkaloid saponins depending on the structure of the aglycones, where steroids themselves in modern clinical studies have roles as agents anti-inflammatory and analgesic.11 Flavonoids in binahong extract work by inhibiting the action of the enzyme cyclooxygenase (COX), thereby reducing the production of prostaglandins by arachidonic acid. Furthermore, neutrophil degranulation will be hampered by a decrease in prostaglandins that inhibit the release of cytokines, free radicals, and enzymes that play a role in inflammation.

Conclusions

There is an effect of subgingival gel extract of binahong (*Andredera cordifolia* (Ten.)

Steenis) 3% on decreasing IL-6 levels of gingival crevicular fluid and improvement of periodontal status in patients with chronic periodontitis.

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