ORIGINAL ARTICLE

Analysis of the Expression of Macrophage among Periodontitis Rat Model after Treatment with *Graptophyllum Pictum (L.) Griff.* Leaves Extract Gel

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ABSTRACT

Introduction: Aggressive periodontitis has the characteristics of rapid loss of periodontal tissue and bone destruction resulting in tooth loss. *Graptophyllum pictum (L.) Griff.* is widely used as herbal medicine in Indonesia. The flavonoid content in *Graptophyllum pictum (L.) Griff.* is known to have a role as an anti-inflammatory and anti-oxidant. This research aimed to analyze the role of *Graptophyllum Pictum (L.) Griff.* extract gel on the amount of macrophages as an inflammatory indicator on periodontal tissue of Wistar rats with periodontitis. **Methods:** Periodontitis was produced in Wistar rats by induced of 2 ml 109 CFU *A. actinomycetemcomitans* at gingival sulcus of the upper right second molar, afterward were treated with 7.5%, 15%, and 30% *Graptophyllum Pictum (L.) Griff.* extract gel for 3 days. Gingival tissues were removed for Hematoxylin Eosin staining for histopathological analysis and measurement of the number of macrophages. **Results:** *Graptophyllum Pictum (L.) Griff.* extract gel at concentrations of 7.5%, 15%, and 30% could significantly decrease the number of macrophages, but only group with a concentration of 15 and 30% can reduce the number of macrophages to reach an amount equivalent to the level in the negative control group. A concentration of 30% extract gel could reduce the number of macrophage cells more than the other two treatment groups. **Conclusion:** The concentration of 30% *Graptophyllum Pictum (L.) Griff.* extract gel was the most effective concentration in decreasing the amount of macrophages.

Keywords: *A. actinomycetemcomitans*, periodontitis, *Graptophyllum Pictum (L.) Griff.* leaves extract gel, macrophage

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INTRODUCTION

Aggressive periodontitis is an inflammation process that cause the severe and progressive destructions of periodontal tissue, as periodontal ligament and alveolar bones. This process caused by specific microorganism (1). One of the periodontal disease that often occurs at a young age is aggressive periodontitis (2). Armitage (3) stated that in some patients with classical localized aggressive periodontitis, 'certain strains and clones of *A. actinomycetemcomitans* appear to be important members of the pathogenic subgingival biofilm' (p.82). In inflammatory conditions, the role of macrophages is to direct the body's defenses in overcoming microorganism infections. Macrophages in inflammatory conditions have a protective function, the amount of macrophages increases in chronic inflammation and population of macrophages resulted in bone resorption (4).

The comprehensive treatment aim of aggressive periodontitisis is to stabilize peridontal health, consist of medical and surgical treatments (5). The phase of treatment in chronic and aggressive periodontitis is not much different, but has the same treatment goals (6). Ramesh et al. (7) stated about herbs as antioxidant source for periodontitis therapy 'Various antimicrobials and chemotherapeutic agents, such as chlorhexidine, triclosan, cetylpyridinium chloride, have been tried and tested in the management of periodontal diseases. Due to its multifactorial etiology and complex disease process, the treatment of periodontitis is still a formidable task to dentists. Therefore, herbal remedies have been sought to achieve antimicrobial, antioxidant, antiseptic, anti-inflammatory, and anti-collagenase effects' (p.1). One of the herbal medicines that is often used by Indonesian people is *Graptophyllum pictum (L.) Griff.* leaf as a material to heal cut, wound, and kind of swellings, and also for the treatment of ulcer, abscess, hemorrhoids (8). Research by Jiangseubchatveera et al. (9) found that components contained in *Graptophyllum pictum (L.) Griff.* leaves were flavonoids steroids, tannins, coumarins, saponins, anthraquinones, phenolics, and

sugars.

Anti-inflammatory and anti-oxidant activity of *Graptophyllum pictum (L.) Griff.* leaves are associated with the presence of high concentration of flavonoid content. The quercetin compound is the main flavonoid content identified in *Graptophyllum pictum (L.) Griff.* leaves (10). According to the components of *Graptophyllum pictum (L.) Griff* . leaves, it is expected that the *Graptophyllum pictum (L.) Griff.* leaves can be used as an anti-inflammatory, especially in aggressive periodontitis. This study analyzed the expression of macrophages in aggressive periodontitis Wistar rats treated with *Graptophyllum pictum (L.) Griff.* leaves extract gel in order to be used marker of inflammation.

MATERIALS AND METHODS

Samples

Twenty six male Wistar rats (*Rattus novergicus*) with the age of 2-3 months and weight of 100-150 grams were used in this experiment. The Wistar rats were randomized into 5 groups, consist of negative control groups (n=4), positive control group (n=4), and 3 treatment groups (n=6). The animals were housed for acclimatization, one week before the start of the experiment. The research was carried out in the Laboratory of Biochemistry, Faculty of Medicine, Universitas Airlangga for maintenance and treatment of experimental animals. Macrophage cells examination from Wistar rat gingival tissue was conducted at the Research Center, Faculty of Dental Medicine, Airlangga University. The study was conducted after approval from the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga.

The production of *Graptophyllum pictum (L.) Griff* leaves extract gel

Graptophyllum pictum (L.) Griff. leaves extract was obtained by maceration of 1 kilogram of *Graptophyllum pictum (L.) Griff.* leaves powder dissolved in 1500 milliliter of 90% ethanol. *Graptophyllum pictum (L.) Griff.* leaves powder was obtained and processed at UPT Materia Medica Batu, Malang, Indonesia. To made leaves extract gel 7.5%, 15%, and 30%, the leaves extracts weighing 3.75 mg, 7.5 mg, and 15 mg were dissolved in a portion of water and then heated at 50°C. Na-CMC was added as much as 50 mg and stirred until homogeneous (11), and then applied topically to the gingiva of Wistar rats once a day for three days, starting after aggressive periodontitis established.

Procedure

After acclimatization for one week, negative control groups received 0.9% NaCl solution and aquadest. All of the animals of positive control group and treatment group were induced by 0.2 ml 109 CFU *A. actinomycetemcomitans* at the upper right second molar region for three times with two-day intervals until aggressive periodontitis developed, characterized by inflammation, redness of the gingiva, and pocket formation (12), afterward treatment group 1, 2, and 3 received 7.5%, 15%, and 30% *Graptophyllum pictum (L.) Griff.* extract gel, respectively (13). *A. actinomycetemcomitans* was obtained from Research Center, Faculty of Dental Medicine, Universitas Airlangga. After the treatment for three days, anesthesia was performed according to the method used by Ionel et al. (14) as follows 'general anesthesia was achieved through intramuscular injection with a solution of Ketamine 10% and Xylazine 2% (2: 1), 0.12 ml / 100 g body weight' (p.91). The mandibular was taken and then was fixed using a 10% formalin buffer solution for 24 hours. After complete fixation, the gingival tissue processing was performed including dehydration, clearing, infiltration in paraffin, embedding, and afterwards, gingival tissue staining was performed using Hematoxylin and Eosin (HE) (Park) and examined for descriptive histology under an 400x magnification using a Nikon H600L light microscope with a 300-megapixel DS Fi2 digital camera and Nikon Imaging System image processing software.

Statistical analysis

The data were carried out distribution test using Kolmogorov Smirnov, homogeneity tests using the Levene's test of Homogeneity of Variance, followed by One-way ANOVA test to find out the difference in the study groups. Data that were normally distributed continued with Tukey's HSD Post Hoc Test to find out the differences in each groups, statistical significant were defined by a $p < 0.05$.

RESULTS

Figure 1 showed the expression of macrophage cells (black arrows) of the subjects with aggressive periodontitis using Hematoxylin-Eosin staining technique and 400x magnification.

Hematoxylin-Eosin staining was conducted to determine the presence of macrophage cell in gingival tissue. The lowest number of macrophage cells were in the negative control group, which was a group that reflected the number of macrophage cells in normal condition. The number of macrophage cells in the negative control group was significantly lower from the positive control group (a group that reflected periodontitis rats without treatment) and treatment group 1 (a group of periodontitis rats that received treatment with 7.5% *Graptophyllum pictum (L.) Griff.* extract gel). These results showed that periodontitis increased the number of macrophage cells, whereas therapy with 7.5% *Graptophyllum pictum (L.) Griff.* extract gel had not been able to reach the number of macrophages under normal condition. The number of macrophage cells in the positive control group was statistically higher than a ll the treatment groups that received *Graptophyllum pictum (L.) Griff.* extract gel

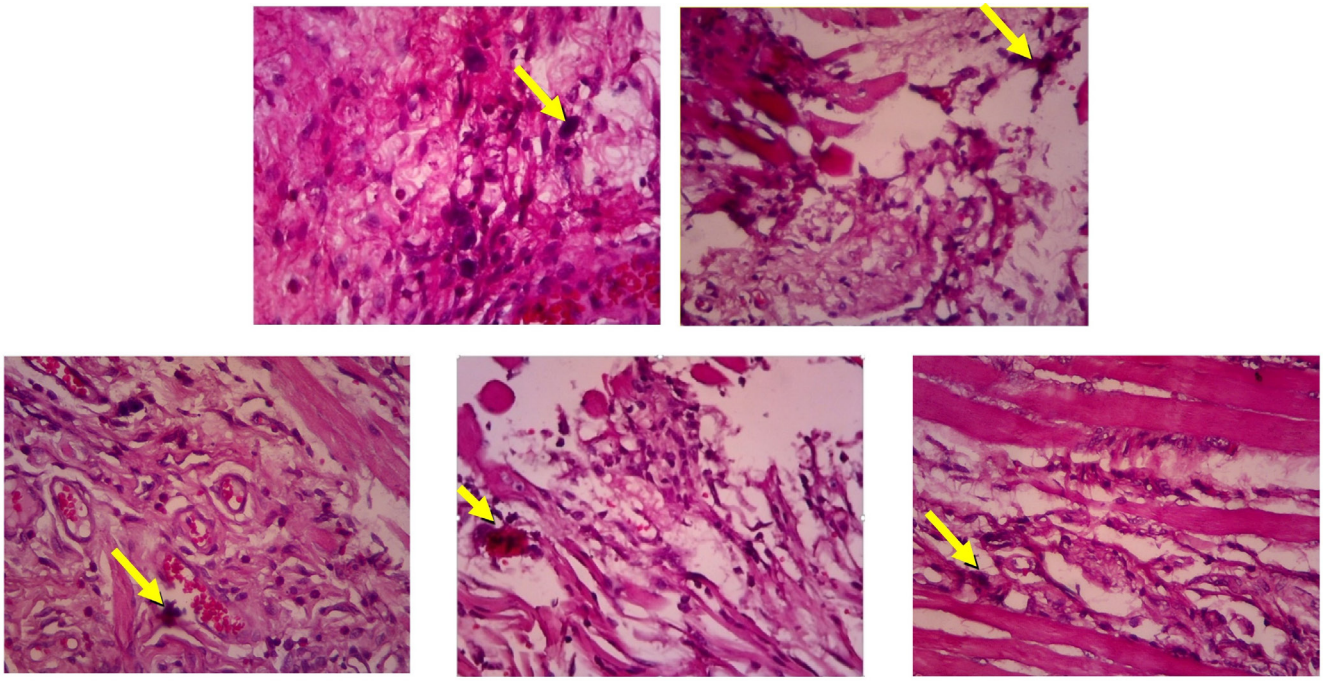

Figure 1: Histopathology examination of macrophages (yellow arrows) from gingival epithelium of Wistar rat using Hematoxylin-Eosin-400x Magnification. A: Negative Control Group; B: Positive Control Group; C: Treatment Group 1; D: Treatment Group 2; E: Treatment Group 3

Table I: The number of macrophages ($\bar{x} \pm SD$), Significance of normality test, and Significance of the one-way ANOVA from gingival epithelium of Wistar rats with periodontitis

Group	n	$\bar{x} \pm SD$	Signicance of the normality test	Signicance of the one-way ANOVA test
Negative control group	4	1.50 ± 0.577	0.846	
Positive control group	4	10.25 ± 2.062	0.859	
Treatment group 1	6	5.00 ± 2.530	0.990	0.000
Treatment group 2	6	3.67 ± 1.211	0.986	
Treatment group 3	6	2.50 ± 1.975	0.787	

Table II: Significance (*p* value) of mean of macrophage number between the groups by Tukey's HSD Post test test.

Group	Negative control group	Positive control group	Treatment group 1	Treatment group 2	Treatment group 3
Negative control group	-	0,000*	0.008*	0,085	0.414
Positive control group		-	0.000*	0.000*	0.000*
Treatment group 1			-	0.228	0.030*
Treatment group 2				-	0.289
Treatment group 3					-

*: There were significance difference

with a concentration of 7.5, 15, and 30% (Table I). Data from the treatment group showed that only treatment group 1 and 3 were statistically different, the number of macrophages of treatment group 3 was lower than tretament 1. Whereas administration of 7.5 and 15% *Graptophyllum pictum (L.) Griff.* extract gel had the same effect on the number of macrophage cells, as well as concentrations of 15 and 30% also giving the same effect (Table II).

DISCUSSION

Graptophyllum pictum (L.) Griff. is a type of vines that are widely used as herbal medicine to cure various diseases, such as anti-inflammatory (8), anti-oxidant (9), antibacteria (10), antipyretic (13), photoprotective (15). It is known that the main flavonoid content of *Graptophyllum pictum (L.) Griff.* is quercetin, a class of flavonoids that is known to have anti-inflammatory and anti-oxidant activity (8, 9). Recent research (16) indicates that some metabolic variations due to flavonoid could decrease the pro-inflammatory effect caused by LPS and IFN- γ, it means that flavonoids increase immunomodulatory activity.

The use of ethanol for the extraction of *Graptophyllum pictum (L.) Griff.* in this study was consistent with research by Poh-Yen et al. (15) which showed that ethanol is a solvent of choice that had better stability for *Graptophyllum pictum (L.) Griff.* Hematoxylin and Eosin (HE) stains used in this research have been used for at least a century and are still essential for recognizing various tissue types and the morphologic change (4).

In this research, administration of *Graptophyllum*

pictum (L.) Griff. extract gel at concentrations of 7.5%, 15%, and 30% resulting in the number of macrophages of all treatment groups were lower than the positive control group, but only a concentration of 15 and 30% indicated the number of macrophages which were statistically the same as normal. This results showed that therapy with *Graptophyllum pictum (L.) Griff.* extract gel at all concentrations able to reduce the number of macrophages, however there was no difference in the number of macrophage cells between the negative control group and treatment group 2 and 3 indicating that only the treatment using *Graptophyllum pictum (L.) Griff.* extract gel at concentration of 15 and 30% were able to reduce the number of macrophages reaching normal condition. The results showed that *Graptophyllum pictum (L.) Griff.* gel administration could reduce the number of macrophages in proportion with an increase in *Graptophyllum pictum (L.) Griff.* gel concentration. Polyphenol content in *Graptophyllum pictum (L.) Griff.* gel, especially quercetin, considered has anti-inflammatory properties as well as non-enzymatic anti-oxidants as an chain breaking anti-oxidant that could inhibit the formation of free radicals, thus inhibit cell membranes damage.

The presence of macrophages in periodontal inflammation has been proven by many studies. Although this study did not distinguish between M1 and M2 macrophages, this study was consistent with the research by Yang et al. (17) which showed that periodontal inflammation is associated with an increase in M1/M2 macrophages ratio and this could be used as valuable information for health status periodontal tissue. Research by Ramesh et al. (7) showed the importance of several herbs in the treatment of periodontal disease, specifically as an anti-oxidant in periodontal disease to overcome the oxidative stress in periodontal disease, and it did not rule out the role of herbs as an anti-inflammatory, especially those related to macrophages. Kuzenko et al. (4) also showed that although macrophages function protectively and were present in acute and chronic periodontal tissue inflammation, the research also showed that macrophage populations in chronic inflammation result in bone resorption.

So far, there had not been much research on the potential of *Graptophyllum pictum (L.) Griff.* in dealing with inflammation in periodontal disease. This research was in accordance with the study of Prasetya et al. (18) also showed that administration of mangosteen peel extract which had the same flavonoid content with *Graptophyllum pictum (L.) Griff.* leaves as much as 60 and 30 mg / kg BW in mice induced periodontitis was able to reduce the infiltration of inflammatory cells. Research by Tjahjani et al. (10) showed that administration of *Graptophyllum pictum (L.) Griff.* leaves ethanol extract at a dose of 300 mg / kilogram BW for 7 days could significantly reduce TNF-α and NO levels in Swiss mice infected with *S. aureus*.

In this research, the decrease in the number of macrophages after the administration of *Graptophyllum pictum (L.) Griff.* might be caused by the content of flavonoids which act as anti-bacterial, anti-inflammatory, anti-oxidant, and immunomodulatory properties.

CONCLUSION

The conclusion of this study was that the administration of *Graptophyllum pictum (L.) Griff* leaves extract gel with a concentration of 30% for 3 days was the most effective in reducing the number of macrophages in Wistar rats model with aggressive periodontitis.

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