

The Effect of Purple Leaf Extract (*Graptophyllum pictum* L. Griff) to The Amount of Fibroblast in Gingiva Rat Wistar induced by *Porphyromonas gingivalis*

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Online submission: 20 Januari 2021

Accept submission: 25 Januari 2021

ABSTRACT

Background: The prevalence of periodontal disease ranks second in dental and oral diseases after caries. Periodontitis is inflammation with bacteria infecting the host and involving all parts of the periodontal tissue. If this condition is left untreated, it can lead to fibrosis and irreversible damage. Various types of periodontitis therapy have not been able to provide optimal results in healing periodontitis and that therapy can cause side effects. Because of this background, the researchers wanted to carry out research on alternative treatments for periodontitis with purple leaves as an anti-inflammatory with an indicator of an increase in the number of fibroblast cells in inflamed areas. The use of purple leaves has been used since ancient times for the treatment of wounds and inflammation.

Objective: To determine the effect of purple leaf extract (EDU) on increasing the number of fibroblast cells in the gingiva of Wistar rats infected by *Porphyromonas gingivalis* (Pg). **Methods:** 30 Wistar rats were divided into 5 groups, namely the normal group (KN), the control group Pg induced (K +) and the treatment group using EDU 2.5% (P1), EDU5% (P2), EDU10% (P3). All groups were induced by Pg except KN. EDU administration once a day for 7 days. On the 7th day the rats were decapitated and their gingivae were taken to make preparations and HE staining was carried out. Results readings in 3 different viewpoints were averaged and analyzed by one-way ANOVA. **Results:** The results showed that the K + group, 2.5% EDU, 5% EDU and 10% EDU groups increased the number of fibroblast significantly when compared to the Pg group ($p < 0.05$). **Conclusion:** EDU can increase the number of fibroblasts in Pg-induced mice

Keywords: Extract of *Graptophyllum pictum* L. Griff leaves, fibroblast, *Porphyromonas gingivalis*.

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INTRODUCTION

Based on National Basic Health Research (RISKESDAS) in 2018, periodontal disease ranks second in dental and oral diseases suffered by many Indonesians with a prevalence of 74,1% [1]. Periodontitis is one of the periodontal diseases that can cause inflammation because bacteria infect the host and cause damage to all parts of the periodontal tissue. *Porphyromonas gingivalis* bacteria (*P. gingivalis*) is one of the microorganisms causing periodontitis.²

P. gingivalis bacteria invade the host and then damage the extracellular matrix, modulating both the inflammatory response and the immune response of the host.³ At the time of inflammatory process occurs fibroblast cell production is increased compared to normal circumstances. Fibroblasts will respond to inflammation by proliferation of inflammatory areas. Inflammation that occurs for a long time and is not given therapy can cause irreversible damage. To prevent the severity of the disease can be done therapeutic treatment in patients with periodontal disease.⁴ *P. gingivalis* bacteria invade the host and then damage the extracellular matrix, modulating both the inflammatory response and the immune response of the host.³ At the time of inflammatory process occurs fibroblast cell production is increased compared to normal circumstances. Fibroblasts will respond to inflammation by proliferation of inflammatory areas. Inflammation that occurs for a long time and is not given therapy can cause irreversible damage. To prevent the severity of the disease can be done therapeutic treatment in patients with periodontal disease.⁴

One of the therapy periodontitis is the administration of medicines that are mouthwashes that are anti-inflammatory. Currently has been widely developed mouthwash with medicinal plant basic ingredients that are believed to have minimal efficacy and side effects. Mouthwash is a medicine that has a formula in the form of a

solution and is generally diluted so that it can be used directly in the oral cavity.⁵ Mouthwash containing benzydamine HCl and alcohol, can usually minimize inflammation, but the product has side effects such as numbness, burning sensation or stung in the oral cavity and vomiting nausea so that other alternatives are needed for the treatment of periodontitis that has lower side effects than the chemical mouthwash.⁶

One of the traditional medicinal plants that has been known to be beneficial for health is purple leaves (*Graptophyllum Pictum* L. Griff). Purple leaves are plants with purple leaves and are commonly found along the street or in people's yard because of their beauty. Purple leaf is one of 13 commodities developed by the Director General of Drug and Food Control (DITJEN POM) as the flagship medicinal plant.⁷

The active compounds contained in purple leaves (*Graptophyllum pictum* L. Griff) are flavonoid compounds, tannins, alkaloids, steroids, saponins, alcohol and calcium oxalate. The complex and diverse content causes purple leaves to have an anti-inflammatory effect. One of the content of purple leaves, namely flavonoids can inhibit the path of cyclooxygenase and lipooksigenase in inflammatory processes.⁸ The inhibition is followed by the inhibition of inflammatory mediators so that the healing process of inflammatory tissue becomes faster which is characterized by a slowdown of inflammatory processes and acceleration towards the proliferation phase. Another content is alkaloids that work as anti-inflammatory by inhibiting the formation of prostaglandins [9]. The safety of purple leaves has been proven by some researchers and proven through toxicity tests conducted over 20 days that the administration of purple leaf extract is not toxic to the hematological profile of male white mice.¹⁰

Because of this background, researchers wanted to conduct research on alternative treatment of periodontitis with purple leaves that have lower side effects than chemical drugs. In addition, the use of purple leaves in the field of Dentistry is also still lacking so researchers want

to prove whether purple leaf extract can work as an anti-inflammatory with indicators of increasing the number of fibroblast cells in the gingiva area that experience inflammation.

Some previous research that has been done Kurniawati (2016), explained that purple leaf ethanol extract (*Graptophyllum pictum* L. Griff) can affect the function of phagocyteocytes exposed *Candida albicans*.¹¹ The study used several concentrations of purple leaf extract which is 2.5%, 5% and 10%. Researchers want to conduct research on whether purple leaves with such concentrations can work as an anti-inflammatory with indicators of increasing the number of fibroblast cells in areas that experience inflammation.

RESEARCH METHODS

This research is experimental laboratory type, with treatment in the form of induction porphyromonas gingivalis and administration of purple leaf extract (EDU). The research was conducted in November 2019 - January 2020. The research was conducted in several places, namely Medicinal Plant Education Tour (WETO) University of Jember to take samples of purple leaves, Center for Development Advanced Science and Technology (CDAST) University of Jember for the manufacture of purple leaf extract, Microbiology Laboratory Faculty of Dentistry University of Jember for the preparation of bacteria *P. gingivalis*, Biomedical Laboratory physiology section of the Faculty of Dentistry University of Jember for the treatment of mice wistar, Laboratory Histology Faculty of Dentistry University of Jember to examine the number of fibroblast cells. The purple leaf used is *Graptophyllum pictum* L. Griff which has been identified by the Indonesian Institute of Sciences (LIPI) through Identification Certificate No. 1110/IPH.06/HM/X/2019 dated October 22, 2019. There were 30 samples each divided into 5 groups namely: normal control group (KN), positive control group (K+) of *P. gingivalis*-induced group (Pg) and treatment group (P1, P2, P3) induced by Pg bacteria only and irrigation

treatment group of purple leaf extract concentration of 2.5%, 5% and 10%. This research procedure has been approved by the Research Ethics Commission of the Faculty of Medicine, University of Jember with the number 633/UN25.8/KEPK/DL/2019.

The research procedure began with the manufacture of purple leaf extract with maceration technique using ethanol solvent 96% and diluted resulting in concentrations of 2.5%, 5% and 10%. Furthermore, the adaptation of male wistar mice for 7 days. After the mice were adapted, followed by the induction of pg bacteria for 2 weeks with the frequency of injection 1 week 3 times so as to create a condition of chronic periodontitis. Induction was performed using a tuberculin syringe with a needle size of 30G on the M1 teeth of the left lower jaw in the entire research group. After clinical symptoms of periodontitis were seen in rats, it was continued at the stage of treatment.

The stage of treatment in pg-induced groups only (K+) is the administration of aquades, in areas with chronic periodontitis. The treatment group was given EDU irrigation concentrations of 2.5%, 5% and 10% as much as 0.27ml. Furthermore, tissue preparation was carried out using ketamine in the entire treatment group until all the mice died which was characterized by a lack of reflexes in the eyes when given light and a heartbeat that was no longer beating. The next procedure is tissue cutting performed on the left regio of the lower jaw ranging from incisiv teeth 1 to molar 2 along with gingival tissue. Cutting on the network is done in the direction of bucco – lingual. Furthermore, the stage of making histology preparations and then done painting HE and observation. Observation and calculation of the number of fibroblast cells is done after histological preparations are formed and carried out using a binocular microscope magnification of 400x. The number of fibroblasts was observed and recorded by calculating the fibroblasts found in three fields of view in each preparation and examined with three different people.

After obtaining the results of the research data, the data is then analyzed using SPSS 16 for windows software. Data is first conducted normality test using Saphiro Wilk test to determine whether the data is normally distributed or not. Furthermore, a variant homogeneity test was conducted to test the sample variation using Levene test. After obtaining normal and homogeneous distributed data, it is continued with one way ANOVA parametric test with a confidence level of 95% ($\alpha = 0.05$). Furthermore, the LSD (Least Significant Difference) test was conducted to find out which group pairs had meaningful differences.

RESEARCH RESULTS

The observations showed semi-ovoid and ovoid fibroblast cells and dark-colored cell nuclei (Figure 1). The results of the calculation showed the number of fibroblast cells in the KP group of 10% namely fibroblast cells that were treated in the form of irrigation of purple leaf extract concentration of 10% had the highest number of cells compared to the control group and other treatment groups. While the normal group has the lowest number of fibroblast cells. The calculation results of each group can be seen in Figure 1. The average number of wistar rat fibroblast cells in each group is presented in Table 1 and Figure 2.

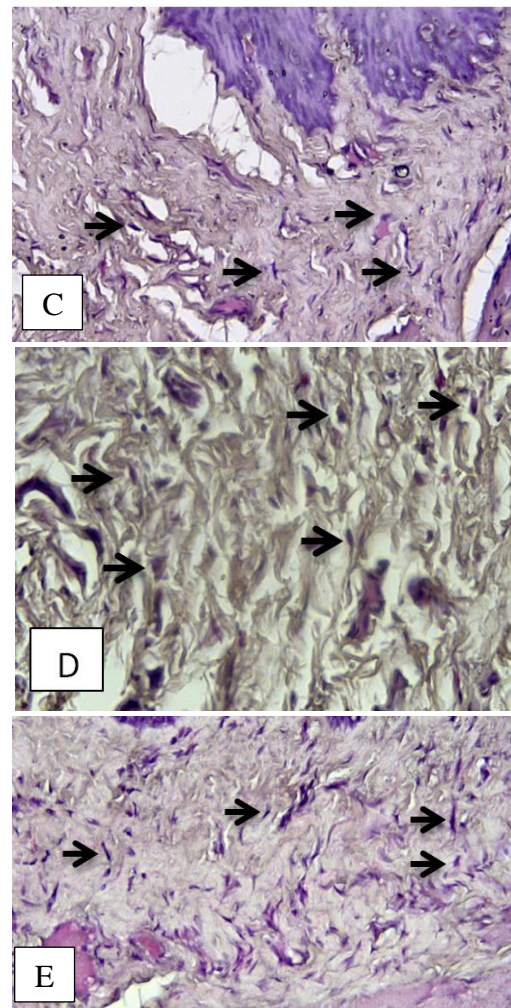
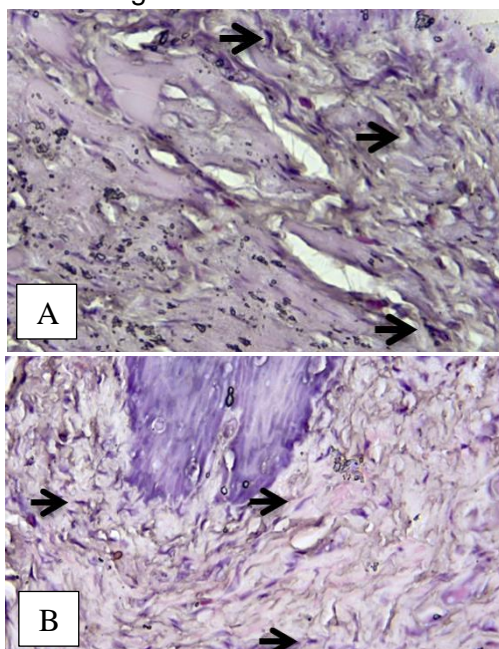


Figure 1. Overview of fibroblast cells in each research group

Description: A. Normal Group, B. Positive Control Group, C. Purple Leaf Extract Treatment Group 2.5%, D. Purple Leaf Extract Treatment Group 5% and E. Purple Leaf Extract Treatment Group 10%. The black arrow indicates fibroblast cells (400x magnification).

Table 1. Average number of fibroblast cells

Research Group	Average	Std.Deviation
KK(-)	128, 50	12, 069
KK (+)	145, 75	7, 890
KP 2,5%	152, 00	2, 380
KP 5 %	167, 25	8, 421
KP10%	208, 50	18, 120

Description :

Std.Deviation : Standart deviation

KK (-) : Normal Group

K (+) : Positive Control Group

KP 2,5% : Treatment Group 2.5%

KP 5% : Treatment Group 5%
KP 10% : Treatment Group 10%

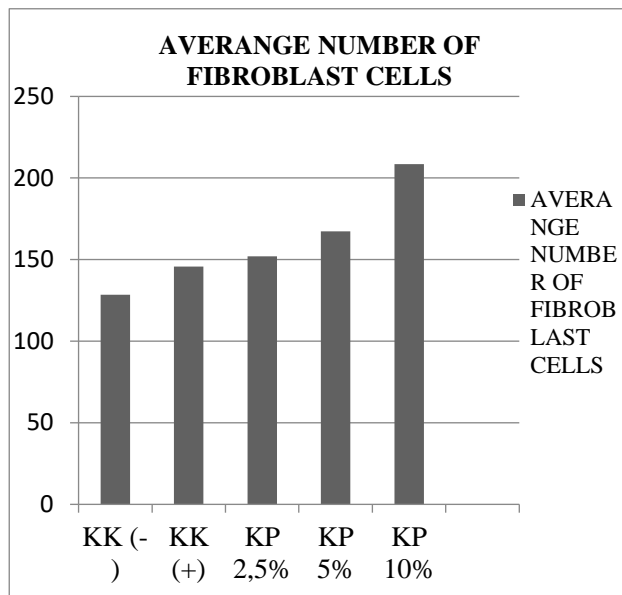


Figure 2. Diagram of the average number of fibroblast cells

Description :

KK (-) : Normal Group
K (+) : Positive Control Group
KP 2.5% : Treatment Group 2.5%
KP 5% : Treatment Group 5%
KP 10% : Treatment Group 10%

One Way ANOVA test results obtained a value smaller than 0.05 ($p < 0.05$) which means there are significant differences in the entire research group. This suggests that there are significant differences in the number of fibroblast cells in the entire research group. The Least Significant Difference test showed that there were significant differences in the entire research group, except between the purple leaf treatment group concentration of 2.5% and the positive control group, the purple leaf treatment group concentration of 2.5% and the purple leaf treatment group concentration of 5%.

DISCUSSION

Purple leaves are chosen for their ability to affect the function of inflammatory cells. One of the anti-inflammatory effects is the inhibition of inflammatory processes and is expected to accelerate the proliferation phase characterized

by an increase in the number of fibroblasts. Based on the results of the One Way ANOVA test, there were significant differences in the entire research group, with the average number of fibroblast cells in the treatment group 2.5%, 5% and 10% greater than the control group. The results showed that the administration of purple leaf extract can increase the number of fibroblast cells, which is suspected because there are various active compounds in purple leaves that are anti-inflammatory. This anti-inflammatory effect will speed up the healing process so that it can be interpreted that purple leaf extract has the potential to be used as an alternative treatment for periodontitis disease. This is in accordance with Rustini's research (2017) which states that purple leaves have an active compound that acts as an anti-inflammatory, namely flavonoid compounds, tannins, alkaloids, sitosterol, glycosides and saponins that are useful as anti-inflammatory.¹² In addition, Andiyani et al (2015) reported that open wounds on the skin of mice given purple leaf extract dried up faster than the comparison group. Clinically dry wounds indicate the healing process of wounds.¹³ The process of healing wounds on the skin is not much different from that that occurs in the mucosa of the oral cavity.

The largest compound content in purple leaves is alkaloids as much as 65,480 mg / g and flavonoids as much as 54,043 mg / g. Alkaloids act as anti-inflammatories by suppressing the release of inflammatory mediators secreted by *P. gingivalis* bacteria through LPS. Other studies have stated that alkaloids act as anti-inflammatory by inhibiting the formation of prostaglandins, suppressing the release of histamine by mast cells, reducing the secretion of IL-1 by monocytes and PAF in platelets.¹⁴ Barriers to the formation of prostaglandins will thus inhibit the work of arachidonic acid.¹⁵ Arachidonic acid is the initial stage of inflammatory occurrence that will release into the tissues. Arachidonic acid will be metabolized through two pathways namely COX and LOX pathways that will produce inflammatory products, one of which is prostaglandins.¹⁶

When prostaglandins are inhibited then fibroblasts that were originally low in proliferation during the inflammatory process, there will be an increase in the number. This is thought to occur because flavonoids inhibit prostaglandins which can cause macrophages to produce growth factors such as PDGF, FGF, TGF- β that will induce fibroblasts to proliferate on inflammatory areas. Next, fibroblasts will produce an extracellular matrix, primary collagen, and fibronectin that will close the wound.¹³

The results of the LSD test explained that the difference in the number of fibroblast cells in the normal group compared to the positive control group did not have a meaningful difference, which means that the two groups had the same effect on the healing process of inflammation. This is because the research was conducted for 7 days and the repair process conducted by fibroblast cells in the control group has not worked to the maximum so that the number is not much different from the normal group. It is shown from the results of table 1 the number of differences between fibroblast cell KN and KK (+) is not far linked, but the number of fibroblast cells in KK (+) is more than KN because the repair process performed by fibroblast cells will occur when the condition of inflammation.

P. gingivalis bacteria will secrete lipopolisakarida (LPS) and LPS will produce glycoprotein such as chemokine and interleukin which can cause inflammation [17]. When inflammation occurs, inflammatory cells such as macrophages will migrate to the inflammatory area to facilitate bacterial lysis and repair. This is in accordance with Rustiasari's statement (2017) which states that macrophages play a role in the process of repairing damaged tissues by secreting various pro-regeneration molecules, namely growth factors that can increase the proliferation of fibroblast cells [18]. So it can be said that even in the kk(+) group experienced inflammation and was not treated, fibroblast cells remained higher than normal due to inflammatory processes.

LSD test results showed that KP 2.5%, 5% and 10% showed meaningless differences. This means that the three EDU concentrations have the same effectiveness in increasing the number of fibroblast cells. It is thought that the content and composition of purple leaf extract such as flavonoids, alkaloids, tannins etc., in addition to having anti-inflammatory effect also has antibacterial effect. EDU is known to effectively inhibit the adhesion of *P. gingivalis* in neutrophils.¹⁹ Indirect effects as an antibacterial against this *P. gingivalis*, causing the amount of *P. gingivalis* in gingival sulcus to decrease. A decrease in the number of bacteria will inhibit the occurrence of inflammatory processes that can lead to an increased number of fibroblasts.

The increase in the number of fibroblast cells in this study is thought to be due to the presence of flavonoid content in purple leaves. Purple leaves can increase immunity (immunomodulatory) and decrease existing inflammation. Increased immunity will stimulate the increase of fibroblast growth factor that plays a role in the proliferation of fibroblast cells, so the number of fibroblast cells will increase as well.²⁰

KP 2.5% compared to KP 5% has no meaningful difference. In Table 1 can be seen from the average number of fibroblast cells between the two groups is not far apart so it can be said that purple leaf extract concentration of 2.5% and purple leaf extract concentration of 5% has wound healing and anti-inflammatory activities that do not differ significantly. This is because the difference between the two concentrations is not far away. This is reinforced by the research of Putri, et al (2017) reported that the difference in concentration is not too much causing results that do not differ significantly to the number of fibroblast cells.²¹ In addition, it can also be caused by a solution whose difference in concentration is not far apart means relatively diluted, so the molecules of chemical compounds are large and the penetration of extracts into the tissue becomes difficult. This can affect the reepithelization process and the number of fibroblasts in the wound healing process.

KP 2.5% and KP 5% compared to KP 10% have a meaningful difference. It can be seen in Table 1 that the average number of fibroblast cells of the purple leaf extract group is 10% higher than the purple leaf extract group 2.5% and purple leaf extract 5%. This is thought to be because the concentration of purple leaf extract concentration of 10% contains the most active compounds compared to purple leaf extract concentrations of 2.5% and 5%. Increased active substances led to increased proliferation of fibroblast cells in the higher concentration treatment group.²² Fibroblasts more actively synthesize extracellular matrix components in response to the presence of inflammation by proliferation and increasing the activity of fibrogenesis so that fibroblasts can be said to be the main agent in wound healing.²² Based on this research, it can be said that along with the increase in concentration, the healing effect is further strengthened by the research of Atmajaya, et al (2019) which reports that the higher the concentration of cork fish extract, the higher the effect on wound healing and the number of fibroblast cells.²³

EDU concentrations of 2.5%, 5% and 10% can increase the number of fibroblasts. The increase in fibroblast cells in this study cannot be said to be fibrosis although the number of fibroblast cells in purple leaf KP concentration is 10% much higher than the number of fibroblast cells under normal circumstances. This reason is because the proliferation phase will occur for 3-14 after inflammation occurs and the peak occurs on the 7th day. After the proliferation phase ends, it will be continued by the maturation phase. The maturation phase begins on the 21st day after inflammation occurs. In that phase there is a decrease in fibroblast cells and excessive collagen produced by fibroblast cells will be gradually degraded by the enzyme collagenase so that there will be a balance between the process of cell proliferation, collagen synthesis and collagen degradation and extracellular matrix.²⁴ It also caused a deficiency in this study because the research time is less long so that the healing process has

not been seen until the number of fibroblast cells returns under normal conditions.

CONCLUSION

Based on the results of studies that have been done can be concluded that purple leaf extract can increase the amount of fibroblasts in wistar rat gingiva induced by *Porphyromonas gingivalis* bacteria.

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