

## ORIGINAL ARTICLE

# Effect of Purple Leaves Extract on Gingival Collagen Density in Wistar Rats Induced by *Porphyromonas Gingivalis*

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## ABSTRACT

**Introduction:** Periodontal inflammation is one of the oral infections caused by *Porphyromonas gingivalis* (*P. gingivalis*). *P. gingivalis* produces endotoxin causing inflammation marked by the degradation of collagen. In cases of severe inflammatory periodontal disease, additional Non Steroid Anti Inflammatory Drugs (NSAID) is needed. But, on the other side, NSAID has side effects. Herbal medicine known can reduce the side effects. One of the herbal medicines was purple leaves (*Graptophyllum pictum* Linn.Griff), that purple leaf extract (PLE) was useful as an anti-inflammatory. It is anticipated that PLE will boost collagen density and lessen inflammation. **Methods:** The experiment design was post-test only control group. Rats were inducted with *P. gingivalis* 0.05ml for the first, fourth, and seventh days to make them suffer periodontal inflammation, 30 male Wistar rats were divided into 6 groups. K- (*P. gingivalis*), KN (Normal group), K+ (*P. gingivalis* + Benzzydamin HCl), K2,5% (*P. gingivalis* + PLE 2,5%), K5% (*P. gingivalis* + PLE 5%) and K10% (*P. gingivalis* + PLE 10%) after treatment on day 8, all groups were sacrificed and made preparations for the gingival collagen to stain with Mallory's Trichrome. Collagen density was measured by the Adobe Photoshop application. **Results:** The result showed that giving PLE 2,5%, 5% and 10% could increase collagen density in Wistar rats significantly ( $p < 0,05$ ). The mean of increased collagen density were 2,5%, 5% and 10% were 119, 120, and 123. **Conclusion:** The study showed that PLE can increase collagen density in Wistar rats induced by *P. gingivalis*. Malaysian Journal of Medicine and Health Sciences (2024) 20(SUPP12) 107-113. doi:10.47836/mjmhs.20.s12.17

**Keywords:** Purple leaves, Collagen, Gingiva, Density *P. gingivalis*

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process of inflammation was one of the body's self-defense actually, mechanisms against foreign objects, but if this process continues continuously (chronically) it will damage the tissues actually (4).

## INTRODUCTION

Dental and oral health problems in Indonesia according to the results of Basic Health Research (Riskesdas) in 2018, the percentage was 57.6% (1). Periodontal disease among the Indonesian population in all age groups has highly prevalence, 96.58% (2). Periodontal disease begins with inflammation in the tissue around the teeth and continues to the tissue structures supporting the teeth, namely cementum, periodontal ligament and alveolar bone (3). Inflammation is a response that aims to eliminate the initial cause of cell injury and remove necrotic cells and tissue caused by the damage. The

The main factor causing periodontal tissue abnormalities was plaque microorganisms. *P. gingivalis* was the dominant bacterium in the biofilm, which can penetrate the host, promote the development of periodontal diseases such as periodontitis, and even trigger systemic disease(5). *P. gingivalis* invasion also causes inflammation, which then releases endotoxins and stimulates phospholipids to release arachidonic acid (6). The metabolism of arachidonic acid causes an inflammatory process which results in decreased fibroblast cell proliferation so that collagen production to repair tissue damage becomes low (7). Wound healing parameters can be seen by observing collagen

density because it has an important role in wound healing. Collagen was a key component in the wound healing process. The role of collagen in accelerating wound healing includes triggering protein synthesis, matrix deposition, cell differentiation, angiogenesis, mitogenesis, and cellular migration such as keratinocytes, epithelialization, fibroblasts, monocytes, macrophages, neutrophils, collagenase induction, wound contraction, and platelet aggregation. Increased collagen fibers indicate wound healing (8).

Drug therapy was needed to reduce severe periodontal inflammation (9). Benzylamine HCl as a mouthwash (commonly called Difflam or Benzylamine hydrochloride (HCl) was a non-steroidal anti-inflammatory drug (NSAID) which can inhibit prostaglandin production indirectly by inhibiting TNF- $\alpha$  stimulation of the production of PGE<sub>2</sub> and PGI<sub>2</sub> prostaglandins in gingival fibroblasts. This drug can cause side effects in the form of itching, rashes, swelling or redness of the skin and wheezing (10). In severe periodontitis, other alternatives were needed to reduce the inflammation periodontal tissues using natural ingredients, namely Purple Leaves (*Graptophyllum pictum* L. Griff). Purple leaves was one of the 13 commodities developed by the Director General of Food and Drug Administration (DITJEN POM) as a superior medicinal plant that can be used as traditional medicine (11). Purple leaves plants can be found from the lowlands to the mountains at an altitude of 1,250 meters above sea level. Grows wild in the countryside or planted as an ornamental plant and hedge plant. It grows well in open places exposed to sunlight, in dry or humid climates (12). Based on toxicity tests, oral administration of purple leaves extract once a day for 20 days was not toxic to the haematological profile of male white mice (13). There was phenolic content in purple leaves, such as alkaloids, saponins, tannins, and flavonoids. This compound has been known to have a good role for humans, as an anti-microbial, anti-inflammatory, anti-allergic, anti-tumor and anti-oxidant to speed up the wound healing process which can protect the human body from free radicals (14). The presence of several ingredients in purple leaves was expected to help and accelerates the inflammatory process in the formation of collagen fibers. The use of purple leaves extract in concentrations of 2.5%, 5% and 10% was known to be able to increase the phagocytic activity of monocytes which plays a role in destroying foreign microorganisms and abnormal particles in the body (15). Based on this argument, the author is eager to find out how purple leaf extract, at concentrations of 2.5%, 5%, and 10%, affects gingival collagen density in Wistar rats that have been infected with *P. gingivalis*.

## MATERIALS AND METHODS

### Samples

This type of research was laboratory experimental laboratory. An ethical certificate N0.850/UN25.8./

KEPK/DL/2020 from the University of Jember Faculty of Dentistry's Health Research Ethics Committee. Laboratory experimental was chosen because each sample was given controlled, measurable treatment, and the effect of the treatment was more reliable. The research used a completely randomized design with a post-test only control group design, namely measurements and observations of the variables studied after being treated and the results were compared with observations in the control group (16).

This research procedure carried out 30 male Wistar rats as samples, divide into six groups, with the criteria being male gender, age 1-4 months, and body weight 150-200 grams. The six groups were K- (*P. gingivalis*), KN (Normal group), K+ (*P. gingivalis* + Benzylamin HCl), K2.5% (*P. gingivalis* + PLE 2.5%) , K5% (*P. gingivalis* + PLE 5%) and K10% (*P. gingivalis* + PLE 10%). Male Wistar rats were adapted to a cage environment covered with wire mesh and were given standard food and water for 7 days. The aim is for the experimental animals to obtain uniformity before research is carried out to control the experimental animals.

### Preparation Purple Leaves Extract (PLE)

The place for making purple leaves extract was at the CDAST Laboratory (Center for Development Advance Science and Technology) at the University of Jember. The procedure for making purple leaves extract, for the first to make dry cymplcia powder, then carry out separation and purification using 96% ethanol solvent, followed by the evaporation stage. After that, purple leaves extract concentrations of 2.5%, 5% and 10% were made. Making Benzylamin HCl preparations as a positive control (K+), by calculating the conversion of a human dose to 200 grams of mice, obtained a result of 0.27 ml for one mouse.

### Preparation *P. gingivalis* suspension

Then the *P. gingivalis* bacterial suspension was made in the Microbiology Laboratory, Faculty of Dentistry, Jember University. Making a suspension of *P. gingivalis* bacteria by placing 2 ml of sterile BHI-B solution in a test tube and adding 1 dose of *P. gingivalis* then homogenizing it on a centrifuge, then placing it in a desiccator to create an anaerobic atmosphere, then incubating for 24 hours at a temperature of 37 °C. After that, dilution was carried out by adding sterile distilled water, homogenized using a centrifuge and the concentration was measured at  $2 \times 10^9$  CFU/ml according to the Mc Farland 0.5 standard with an absorbance of 0.05 and a wavelength of 560nm using a spectrophotometer (17).

### Preparation periodontitis model on Wistar rats

Wistar rats were induced with *P. gingivalis* bacteria suspension on the buccal gingiva of the lower left first molar with a dose of 0.05 ml each and administered on the first, fourth and seventh days using a tuberculin syringe with a 30 gauge needle (18). After periodontitis

model on Wistar rat successfully continued with treatment groups. Purple leaves extract (PLE) and Benzylamine HCl were given once a day for 7 days by irrigation on the gingiva of the lower left 1st molar. Purple leaves extract and Benzylamine HCl were given once a day for 7 days by irrigation on the gingiva of the lower left 1st molar. Euthanasia treatment was carried out chemically, using ketamine at a lethal dose. The euthanasia stages include: preparation of the ketamine dose used of 120-150 mg/kg bw with intraperitoneal (IP) injection with a 1 ml syringe and 5-8 inch needle. Position the rat with its head lower than its abdomen. Ketamine is subsequently given at a 45-degree angle to the Wistar rats. Throughout a brief one to five-minute slow down, the euthanasia process is monitored for breathing and heart rate until it stops. (18).

### Preparation and observing of gingival collagen fibers

The animals model periodontitis were decapitated successfully then parts of the lower left jaw which were induced by *P. gingivalis* bacteria were taken. The tissue taken was decalcified to soft tissue. Next, paraffin blocks were made and Mallory's Trichrome staining was then observed using a light microscope with 400x magnification and the density of collagen fibers in three gingival fields was seen and calculated. Collagen density was obtained using the Adobe Photoshop 7.0 application by determining the average of the pixel of blue colour in three different areas. (19).

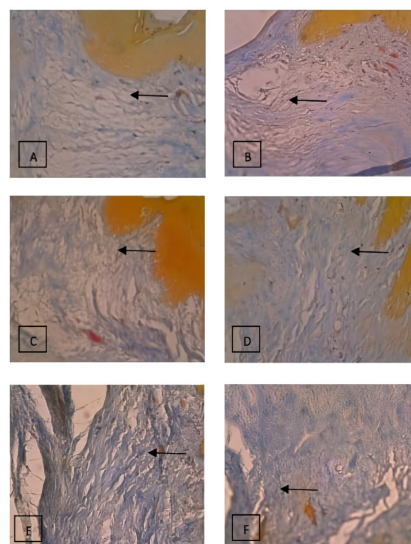
### Statistical analysis

The One Way Anova test was performed to analyze the data and then followed the Post Hoc LSD with significant level at 0.05

### RESULT

This study used 30 male Wistar rats who were treated, divided into 6 groups, after which the animals model periodontitis were decapitated and parts of the lower left jaw which were induced by *P. gingivalis* bacteria were taken. Next, tissue preparations were made using Mallory's Trichrome staining. Using Mallory's Trichrome can provide 3 different stains, carbol fuchsin for nuclear colouring, orange G for cytoplasm and aniline blue for collagen. Light microscope observations to calculate the density of collagen fibers were carried out using 400x magnification with 3 fields of view on the gingiva of Wistar rat teeth. The results of staining with Mallory's Trichrome to observe collagen density at 400x magnification were presented in Figure 1.

The result of the study shows that there were differences in the gingival collagen density of Wistar rats groups. The K- group had the lowest collagen density results, then it increased in KN group, then K+, K2.5% group, K5% group, and the highest collagen density results in Wistar rat gingiva at K10% group (Table I).



**Figure 1:** A histological image of the gingival collagen density with Mallory's Trichrome using a light microscope with 400x magnification. The black arrow represent gingival collagen fibers. A. Negative Control group (K-) : induction *P. gingivalis*, B. Without interference (KN) , C. Positive Control (K+) :(*P. gingivalis* + Benzylamine HCl), D. K2.5% :(*P. gingivalis* + PLE 2.5%) , E. K5% :(*P. gingivalis* + PLE 5%), F. K10% :(*P. gingivalis* + PLE 10%), PLE : Purple Leaves Extract

The research data that has been obtained was tested using the One-way Anova test to determine whether there were meaningful/significant differences or not. The results of the One-way Anova test showed a significance of 0.000, which meant there was a meaningful/significant difference in the results of this study ( $p < 0.05$ ) was shown in Table I.

**Table I. Average and Standard Deviation (SD) of Wistar Rats Gingival Collagen Density (pixel)**

Groups	Gingival collagen density (pixel)	p-value
K-	113.350020 ± 2.38*	0.000*
KN	116.940680 ± 5.42*	
K+	117.666680 ± 7.89	
K2.5%	119.222000 ± 8.41	
K5%	120.433340 ± 12.06	
K10%	123.062660 ± 18.120	

\*The One-Way Anova Test indicates a significant difference within the groups ( $p < 0.05$ )  
 K- : (*P. gingivalis*), KN:(Normal group), K+ :(*P. gingivalis* + Benzylamine HCl), K2.5% :(*P. gingivalis* + PLE 2.5%) , K5% :(*P. gingivalis* + PLE 5%), K10% :(*P. gingivalis* + PLE 10%), PLE : Purple Leaves Extract

Then proceed with the Post Hoc Least Significant Difference (LSD) test to determine whether there were meaningful/significant differences in each group. Based on the results of the Post Hoc Least Significant Difference (LSD) test, it shown that there was a meaningful/significant difference in the gingival collagen density of Wistar rats which was indicated by a significance value of  $p < 0.05$ , namely the K- group against the KN group, K5%, K10%, group KN against K+ group, K25%, K10%, K+ group against K10% (Table I).

## DISCUSSION

There were notable distinctions between the KN and K- groups, according to the LSD test analysis results. In gingival connective tissue under normal circumstances, collagen fibers make up the majority of the biochemical component. Collagen is structured in waves and groups and plays a role in tissue repair by stimulating gingival fibroblast cells, among other things (20, 21). Because collagen is the primary extracellular matrix protein that helps develop scar tissue's strength and serves as a structural supporting for tissue, it has an impact on wound healing (22). The LSD test analysis results demonstrated that the KN and K- groups differed significantly from one another. Collagen fibers, which are structured in waves and groups in gingival connective tissue, are the major biochemical component under normal conditions. Collagen promotes during tissue repair by, for instance, stimulating gingival fibroblast cells (20), (21). Due to its function as the primary extracellular matrix protein that gives scar tissue strength and serves as a structural scaffold for tissue, collagen helps in the healing of wounds (22). Compared to the KN group, the K-group has less collagen fibers. *P. gingivalis* can colonize the oral cavity because of its ability to adhere to surfaces and invade them. These germs will encroach on the body's defenses. These bacteria will invade the body's defence cells using leukotoxins. This bacterium also has cytotoxins which were able to inhibit the formation of fibroblast proliferation so that collagen formation was also reduced (23) This was supported by other research that *P. gingivalis* can induce collagenolysis, the release of collagen from connective tissue so that *P. gingivalis* bacteria can reduce the density of collagen fibers (24)

The K+ group, which received the medication Benzylamine HCl, had more collagen fibers than the KN and K-group, as seen by the significant difference in the LSD test findings. The text Among the non-steroidal anti-inflammatory drug (NSAID) class of topical medications was benzylamine HCl, an anti-inflammatory that was commonly used to treat oral conditions. The body's cyclo-oxygenase enzyme, which generates a variety of substances including prostaglandins that promote inflammation, is inhibited by benzodamine hydrochloride (25). Thus, the medication Benzylamine HCl can prevent the inflammation of the periodontal tissues brought on by the *P. gingivalis* bacteria.

There were significant differences, according to the LSD test analysis results for the K2.5% group with K- and KN, the K5% group with K- and KN, the K10% group with K-, and the KN and K+ groups. Because purple leaf extract contains phenols with anti-inflammatory properties, such as alkaloids, flavonoids, and tannins, there was an important rise in collagen fibers in the PLE group. The tertiary structure of proteins on the surface of bacteria can be manipulated by alkaloids. The hydrophobicity of bacterial cells that interact with the

fimbriae is reduced as a result of this chemical inserting on the hydrophobic side of the protein, which causes clumping of bacterial surface proteins. This protein so loses its hydrophobic shape, which lowers the bacteria's hydrophobicity. Bacterial adherence to host cells will be inhibited by this decrease in hydrophobicity, which will stop hydrophobic interactions of bacterial surface components with host cells (26). The process of bacterial adhesion inhibition prevented tissue harm by preventing the germs from interacting with the host (27). Alkaloids have anti-inflammatory properties as well. Their anti-inflammatory action involves inhibiting or regulating inflammatory mediators like COX-2 in order to minimize the inflammatory process (28).

By preventing the synthesis of prostaglandins from arachidonic acid and other inflammatory mediators like histamine and serotonin, flavonoids were able to bring down the inflammatory process. By blocking the formation of prostaglandins, flavonoids can also suppress the activity of arachidonic acid by blocking the lipoxygenase and cyclooxygenase pathways. This reduces the release of inflammatory mediators and the emigration of leukocytes to the location of inflammation. In order to accelerate the healing process, this can intensify the inflammatory process until it reaches the proliferation stage (29). T cells can be activated by flavonoids, which can then cause them to differentiate and multiply into TH1, TH2, and TH3 cells. Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) is synthesized by TH3 cells and may stimulate fibroblast proliferation, enhancing the formation of collagen fibers (30).

By blocking the enzymes reverse transcriptase and DNA topoisomerase, the tannin content can act as an antibacterial (31). Tannin is a polyphenol that can trigger TGF- $\beta$  for fibroblast proliferation. It can also trigger lymphokines to increase macrophage migration. Macrophages can also stimulate the secretion of growth factors, which in turn increases fibroblast proliferation and collagen fibers production (32).

Other than that, the LSD test findings for the K+ group with 2.5% and K5%, K2.5% with K5% and 10%, and K5% with K10% demonstrated that there were no significant differences. There was little variation in the K+ group between 2.5% and K5% because Benzylamine HCl and PLE 2.5% and 5% have an antibacterial effect and could function as periodontal anti-inflammatories. Benzylamine HCl has the ability to indirectly reduce prostaglandin production by blocking TNF- $\alpha$ -stimulated prostaglandin production in gingival fibroblasts, specifically PGE<sub>2</sub> and PGI<sub>2</sub> (33). Fibroblast proliferation, which was relatively restricted during inflammation, will increase when prostaglandins are suppressed, as will the synthesis of collagen (34). Purple leaf extract, a substance that contains flavonoids and alkaloids, has anti-inflammatory effects as well. As an anti-inflammatory, Benzylamine HCl was consequently

a comparable purple leaf extracts at 2.5% and 5% concentrations.

The LSD test results, specifically in the K2.5% group comparing K5% and 10% and the K5% group comparing K5% and K10%, indicated no significant differences. This indicates that *P. gingivalis*-induced gingival collagen fiber density can be increased in Wistar rats at all three concentrations. Purple leaf extract can be used as an alternative inflammation therapy because it shows signs of being able to increase gingival collagen fiber density to a higher degree than the positive control group. The treatment group that received purple leaves extract had higher collagen density results than the positive control group (Benzzydamin HCl) and had an effect on the negative control group. In the treatment group, it was observed that the higher the 10% concentration of purple leaf extract, the higher the collagen density. This was due to the phenolic content of the purple leaf extract, which includes tannins, alkaloids, and flavonoids, which had a good anti-inflammatory role. Additionally, the collagen assisted in tissue repair during the inflammation process (35).

Proliferation was one stage of inflammation. The process of proliferation starts on the first 48 hours and peaks on day seven, when it reaches its peak. Fibroblasts are one of the cells that are engaged in the process of proliferation. The fibers of collagen in connective tissue are formed by fibroblasts, which are living cells. Extracellular matrix hyaluronic acid, fibrin, fibronectin, glycosaminoglycans, and tropocollagen matrix are among the structural molecules that fibroblasts will grow and create. Thus, the best duration for the formation of collagen is by giving the extract for seven days. This relates to studies by Rahim et al. showing that giving purple leaf extract enhanced the number of fibroblasts on day seven, and by Sitha et al. showing that cassava leaf extract may increase the amount of fibroblasts and collagen density in the gingiva of periodontitis rat models on day seven (36).

## CONCLUSION

Purple leaves extract (*Graptophyllum pictum* (Linn) Griff) have an effect on increasing the density of gingival collagen fibers in Wistar rats induced by *P. gingivalis* bacteria.

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