



Effects of Giving Chinese Petai Leaf Extract Gel (*Leucaena glauca*, Benth) 6% and 15% on the Number of Fibroblasts in Healing Gingivitis of Male Wistar Rats

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ABSTRACT

Gingivitis is one of the most common types of periodontal disease in the world. This disease is caused by bacterial infection in plaque accumulation which releases endotoxins and causes inflammation in healthy gingival tissue. Fibroblast cells play an important role in the proliferation and maturation phases in accelerating the healing of gingivitis because of their ability to stimulate the formation of collagen fibers which will later repair damaged gingival connective tissue. The use of natural ingredients that have the potential to be developed in curing gingivitis, one of which is the administration of petai cina leaves. The content of flavonoids, saponins, tannins and alkaloids in petai cina leaves can have healing potential because it can trigger the proliferation of fibroblasts in the formation of new blood vessels and regeneration of damaged tissue. This study aimed to determine the number of fibroblast cells in induced rat gingival *Porphyromonas gingivalis* ATCC®33277™ after administering 6% and 15% petai cina leaf extract gel. This research method used 40 samples of gingival tissue from the mandibular incisors of male Wistar rats (*Rattus norvegicus*) by following the inclusion and exclusion criteria. The research results are based on tests One Way Anova showed that there was a significant difference in the number of fibroblasts ($p < 0.05$) between treatment groups on day 5 and day 3. showed that no significant differences were found ($p > 0.05$). Concentrations of 6% and 15% showed higher mean fibroblast values compared to the distilled water group and lower than the Gengigel group. This study concluded that 6% and 15% petai cina leaf extract gel had a significant effect on increasing the number of fibroblasts in the gingiva of mice experiencing gingivitis on the 5th day.

Key words: Gingivitis, inflammation, fibroblast, chinese petai leaf

I. INTRODUCTION

Gingivitis is one of the most common periodontal diseases in the world.¹ According to the 2018 Basic Health Research Report (RISKESDAS), the prevalence of gingivitis in Indonesia reached 74%. North Sumatera is a region with a gingivitis prevalence of 15.6%.² The causes of gingivitis are generally divided into two causes, namely the main cause and predisposing causes. The main cause of gingivitis is the buildup of colonies of microorganisms that form dental plaque at the edge of the gums, for example, bacteria *Porphyromonas gingivalis* in dental plaque is the main cause of gingivitis. Predisposing factors for gingivitis include local factors such as cavities, food waste and crowded teeth.^{1,3} If gingivitis is not treated quickly and appropriately, it can develop into periodontitis which is characterized by loss of attachment (loss of attachment) and can cause tooth loss over some time. This can disrupt the balance and function of the oral cavity.^{1,3}

The healing process for gingivitis generally goes through four major stages: hemostatic stage, inflammatory stage, proliferation stage, and maturation stage. The hemostatic and inflammatory phases are characterized by the activity of neutrophils and macrophage cells. The proliferation phase is characterized by the presence of fibroblasts from day 3, reaching its peak on day 5, and the maturation phase is characterized by wound healing. Fibroblasts play an important role in accelerating the healing of gingivitis during the proliferation and maturation stages because they can stimulate the formation of collagen fibers which then build the injured gingival connective tissue.⁴

One of the natural ingredients that can increase fibroblast proliferation in accelerating the healing of gingivitis is Chinese petai leaves. Based on phytochemical results, petai cina leaves contain flavonoids, phenolics, saponins, terpenoids, tannins



and alkaloids which are useful as antibacterial, anti-inflammatory, anti-hyperglycemic, analgesic, antioxidant and larvicide.^{5,6}

Research conducted by Widyantoro and Sugihartini (2015) stated that the tannin and flavonoid content of petai china leaves can stimulate fibroblast proliferation and collagen formation which is effective in tissue regeneration in wound healing.⁷ Other research conducted by Rohmah, et al (2016) compared the effectiveness of healing burns from petai china leaves with castor leaves, showing that petai china leaves had a wound healing time of 2 days faster than castor leaves. This is because petai china leaves contain 6.74% saponin, 7.92% lectin, and 13.34% tannin, which function to increase the formation of new blood vessels and stimulate fibroblast proliferation, thereby speeding up the wound healing process.⁸ Research conducted by Dewantari and Sugihartini (2015) showed that administering 15% and 30% petai china leaf extract gel to guinea pig burns showed an effective healing effect on healing burns in guinea pigs.⁹ Research conducted by Veronica and Dwiastuti (2021) showed that the 6% petai china leaf extract gel formula provided a healing effect in the form of closing cut wounds in mice which achieved 100% results on the 11th day.¹⁰

Chinese petai leaves should be used as an alternative to speed up the healing of gingivitis. However, until now there has been no further research regarding the use of petai china leaf gel on the number of fibroblast cells in the gingivitis healing process. Therefore, researchers are interested in conducting research by comparing petai china leaf gel extract with a concentration of 6% and 15% which is expected to produce good concentration and biocompatibility as an additional treatment to accelerate the healing of gingivitis.

METODE PENELITIAN

The type of research carried out is a true experiment with a research design post-test only controlled group design. Chinese petai leaf collection (*Leucaena glauca*, Benth) was obtained from the Baringan Garden, Tebing Tinggi City. Extract preparation was carried out at the USU Pharmacy Integrated Laboratory. Gel making and preparation evaluation were carried out at the USU Physical Pharmacy Laboratory. Phytochemical screening was carried out at the USU FMIPA Organic Chemistry Laboratory. Adaptation and treatment of experimental animals was carried out at the USU Medical Faculty Pharmacology Laboratory. Histopathological examination was carried out at the USU FK Prospecta Anatomy Pathology Laboratory. The time of the research was

carried out from July to September 2023. The research samples used in this research were male Wistar rats which were kept in the Pharmacology Laboratory of FK USU as many as 40 samples using the method simple random sampling and use a simple research calculation formula. The sample was divided into 8 treatment groups with 5 repetitions. The 8 groups include:

1. Group 1: Chinese petai leaf extract gel 6% day 3
2. Group 2: Chinese petai leaf extract gel 6% day 5
3. Group 3: Chinese petai leaf extract gel 15% day 3
4. Group 4: Chinese petai leaf extract gel 15% day 5
5. Group 5: Gengigel as positive control on day 3
6. Group 6: Gengigel as positive control on day 5
7. Group 7: Aquades as negative control on day 3
8. Group 8: Aquades as negative control on day 5

Simplicia and Extracting Chinese Petai Leaves

Simplicia is made by collecting 3 kg of petai china leaves, then washing them thoroughly using distilled water and drying them in the sun until dry. Chinese petai leaves were weighed as much as 200 grams and put in the microwave for 10 minutes and repeated until all the petai china leaves were used up

Extraction is carried out using multilevel maceration. Dried simplicia is ground with a blender and then sieved with a sieve to produce fine simplicia. Maceration I is carried out by weighing 200 grams of fine simplicia and then placing it in a maceration container. Next, 1.5 liters of 96% ethanol was added and stirred, then left in a maceration container that had been wrapped in aluminum foil to block sunlight for 5x24 hours, while occasionally stirring every 24 hours. After that, the soak is filtered using filter paper, so that filtrate I is obtained and the dregs are then collected. Maceration II is carried out by adding 500 liters of 96% ethanol to the dregs resulting from filtering filtrate I into the maceration container, then stirring and leaving for 3x24 hours, stirring occasionally every 24 hours. After that, filter with filter paper until filtrate II is obtained. Mix filtrate I and filtrate 2 then stir and thicken using a tool rotary vacuum evaporator at a temperature of 90OC, then evaporate in a water bath until a thick extract is obtained.

Chinese Petai Leaf Extract Gel

Gel making begins by making a gel base using carbopol 940 which is developed in hot water



and then stirred with a homogenizer with a speed of 500 rpm which is simultaneously increased to 1036 rpm so that it is completely dispersed and a good gel base is formed, then added triethanolamine little by little and distilled water until a clear gel base is formed, then add propylene glycol as a gel base softener and methylparaben which has previously been dissolved in hot water. The thick extract of petai cina leaves that was obtained was weighed 6 grams to make a gel concentration of 6% and weighed 15 grams to make a gel concentration of 15%. The thick extracts that have been weighed are mixed into the gel base and homogenized with a homogenizer for approximately 15 minutes until a gel is mixed evenly.

Evaluation of Gel Preparations

The gel that has been obtained is followed by an evaluation of the gel preparation to determine the quality and suitability of the gel which includes organoleptic tests, homogeneity tests, pH tests, spreadability tests, viscosity tests, and stability tests carried out at the USU Physical Pharmacy Laboratory.

Preparation and Treatment of Experimental Animals

Experimental animals were divided into 8 treatment groups. Each treatment consists of 5 samples. Samples will be sacrificed on day 3 and day 5. Experimental animals that have been adapted for 7 days are induced with bacteria Porphyromonas gingivalis ATCC 033277 UMBRELLA in the gingival sulcus of the lower incisor as much as 0.02 ml. Animals

that had experienced inflammation were then treated with 6% and 15% petai cina leaf extract gel, a positive control of Gengigel, and a negative control of distilled water. Samples were taken on the 3rd and 5th day of surgery and then made into histological preparations to examine the number of fibroblast cells through histopathological examination.

Histopathological Examination

Preparations of the lower jaw gingiva were made in microscopic preparations by fixing them with 10% formaldehyde and making histological preparations. Next, coloring is done with hematoxylin eosin. The stained histological preparations were then counted for the number of fibroblast cells under an Olympus CX31 microscope with 400x magnification. Calculation of the number of fibroblast cells in each preparation was systematically seen in 3 fields of view.

II. RESULT

The results showed that all data were normally distributed and homogeneous (p>0.05). Test results from one-way ANOVA showed that there was a significant difference in the mean number of fibroblasts on day 5 between all groups (p<=0.05), whereas on day 3 there was no significant difference (p>0.05). Test results after this LSD on the 3rd day after treatment showed that there was no significant difference in the number of fibroblasts between the two different groups (p>0.05). However, it showed a significant difference on day 5.

Table 1. Results of Normality and Homogeneity Tests on Day 3 and Day 5

Table with 5 columns: Group, Day 3 Normalitas, Day 3 Homogenitas, Day 5 Normalitas, Day 5 Homogenitas. Rows include K 6%, K 15%, (+), and (-).

Table 2. One-Way ANOVA Test Results on Day 3 and Day 5 After Treatment

Table with 5 columns: Group, Day 3 Mean±SD, Day 3 p, Day 5 Mean±SD, Day 5 P. Rows include K6%, K15%, (+), and (-).

Description: Test results in one way ANOVA *Significant



Table 3. Test Results After this LSD 3rd Day After Treatment

Group	K 6%	K 15%	(+)	(-)
K 6%	-	0,948	0,653	0,748
K 15%	0,948	-	0,700	0,699
(+)	0,653	0,700	-	0,443
(-)	0,748	0,699	0,443	-

Table 4. Test Results After this LSD 5th Day After Treatment

Group	K 6%	K 15%	(+)	(-)
K 6%	-	0,022*	0,000*	0,000*
K 15%	0,022*	-	0,005*	0,000*
(+)	0,000*	0,005*	-	0,000*
(-)	0,000*	0,000*	0,000*	-

Description: Test results post hoc LSD *Significant

Evaluation Results of Gel Preparations

Organoleptic Test

Organoleptic tests are carried out by visually examining color, smell and taste. The color produced by the two concentrations of petai china leaf extract gel is dark brown. The odor produced by a 15% concentration is a strong herbal odor, while a 6% concentration has a slight herbal odor. The taste produced by a 6% concentration is bland while a 15% concentration has a slightly bitter taste. From these results, the 6% concentration has better test results.

Homogeneity Test

The gel homogeneity test was carried out using object glass to know that the gel was homogeneous. The results show that all particles have dissolved and the color is even, which is included in the good category.

Spreadability Test

This test is carried out on glass plates given different loads. Each load was repeated 3 times. At a load of 50 grams, the spreadability of the 6% concentration gel was obtained with an average of 5.1 cm and the 15% concentration gel with an average of 5.5 cm. At a load of 100 grams, the spreadability of the gel concentration was 6% with an average of 5.5 cm for a concentration of 15% with an average of 6.3 cm. At a load of 150 grams, the spreadability of a gel with a concentration of 6% was obtained with an average of 6.2 cm and a gel with a concentration of 15% with an average of 6.7 cm, which is included in the good category, where the normal spreadability test ranges from 5-7 cm.

Viscosity Test

The gel viscosity test was carried out using a viscometer with results of 23,570 mps for a 6% concentration and 26,767 mps for a 15%

concentration which is included in the good category, namely ranging from 20,000-40,000 mps.

pH Test

The gel pH test was carried out using a pH meter. The results obtained were 4.61 for a concentration of 6% and 4.57 for a concentration of 15% which is included in the good category, where a good gel pH test ranges from 4.5 to 6.5.

Stability Test

The results of the gel stability test showed that the gel preparation was stable as indicated by no changes in shape, color and pH during the 12-day test period.

III. DISCUSSION

The results of the One-Way ANOVA test carried out showed that on the 3rd day, there was a difference but it was not significant, while on the 5th day, it showed that there was a significant difference in the mean number of fibroblasts on the 5th day between all groups ($p \leq 0.05$). This is in line with the research of Agustin, et al, that the neutrophil infiltration process occurs 24 hours to 3 days after inflammation occurs, then continues with the proliferation phase from day 3 until its peak on day 5 and ends on day 7 and then continues in the remodeling on day 7 to day 14. The end of the inflammatory phase and the beginning of the proliferation phase is the beginning of fibroblasts starting to appear in the wound area. Infiltration and degradation of fibrin clots originating from fibroblasts.¹¹ This means that the results of the research on all treatment groups on day 3 did not show a significant difference in the number of fibroblasts. However, it showed an increase in fibroblasts and a significant difference on day 5 where day 5 was the beginning of the peak of fibroblast proliferation.



On the 5th day, it was found that there was an increase in the number of fibroblast cells with the highest to lowest mean value found in the Gengigel group (12.00 ± 0.848), concentration 15% (9.80 ± 1.518), concentration 6% (8.07 ± 0.434), and distilled water (4.80 ± 1.216). When compared, the 6% and 15% groups had a higher mean number of fibroblasts compared to the distilled water group both on day 3 and peak on day 5. This is because the flavonoid content in petai china leaves has proliferative capabilities TGF- β which results in a proliferation process so that the number of fibroblasts increases.⁹ The saponin content in crushed petai china leaves functions to increase the formation of new blood vessels and stimulate collagen formation by fibroblasts.¹² The tannin and flavonoid content of petai china leaves can stimulate fibroblast proliferation and collagen formation which is effective in tissue regeneration in wound healing.⁷

In line with research conducted by Eritriana, et al regarding Chinese petai leaf ointment with a concentration of 15%, it showed better results in healing abrasion wounds than 10%.¹³ Research conducted by Manapode, et al 2016 showed that the process of healing burns in rabbits given 8% lamtoro or petai china leaf extract cream had the fastest effect when compared to concentrations of 2% and 4% because it contained more active substances which could help the burn wound healing process.¹⁴ Research conducted by Fitriana, et al 2018 showed that the vascular counts in the 30% and 45% lamtoro or petai china leaf extract gel treatment groups were significantly different compared to the negative control group, both on days 3 and 5, while the 15% group did not show any significant differences.¹⁵ This shows that the higher the concentration of petai china leaves used, the greater the content of active compounds in them which can affect healing. This is in line with the results of research where a concentration of 15% showed a higher mean fibroblast value compared to a concentration of 6%.

From the results of the research above, the average value of fibroblasts produced after treatment with concentrations of 6% and 15% was not able to compete with Gengigel. Both in the 15% concentration and 6% concentration groups. This may be due to the content of Gengigel which consists of hyaluronic acid with a high molecular weight that can inactivate bacterial hyaluronidase, normalize macroaggregation of connective tissue proteoglycans, and bind with free water to produce anti-edema effects and induce periodontal healing.¹⁶ However, petai china leaf extract gel was still able to influence the increase in fibroblasts in

rat gingivitis, which had a higher mean value compared to distilled water.

Overall, the evaluation of the preparation showed that the petai china leaf extract gel with concentrations of 6% and 15% were in a good category. This shows that the Chinese petai leaf extract gel is suitable to be used as an alternative ingredient to accelerate the healing of gingivitis which can be tested on living creatures, namely in animal trials first before being tested through clinical trials on humans.

IV. CONCLUSION

There was a difference in the number of fibroblasts in healing gingivitis of male Wistar rats after being given 6% and 15% petai china leaf extract gel but it was not significant on day 3 ($p > 0.05$) and significant on day 5 ($p < 0.05$).

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