



## ORIGINAL RESEARCH

# The Effect of Bay Leaf Extract Gel on Increasing Fibroblasts in Traumatic Ulcers Healing Process

*Firawan, Kurnia Nisa Putri<sup>1</sup>; Kusuma, Ira Anggar<sup>1\*</sup>; Istiadi, Hermawan<sup>2</sup>; Santoso, Oedijani<sup>1</sup>*

*1 Department of Dentistry, Faculty of Medicine, Universitas Diponegoro*

*2 Department of Anatomical Pathology, Faculty of Medicine, Universitas Diponegoro*

*\*Corresponding author: iraanggarkusuma@gmail.com*

## KEY WORDS

Bay leaf Extract;  
Traumatic Ulcer;  
Wound Healing;  
Fibroblast Cell

## ABSTRACT

The wound healing process of traumatic injuries in the oral cavity involves four stages: hemostasis, inflammation, proliferation, and remodeling. Fibroblast cells are essential for the wound healing process, appearing on the 3rd day and reaching their peak on the 7th day after injury. This study reveals that bay leaf extract can accelerate wound healing process by mitigating inflammation and enhancing fibroblast cell activity. The type of research is experimental research with post-test only control group design, consisting of control group, treatment groups were given 5% and 10% bay leaf extract gel with 5 samples of male Wistar rats on each group. The application of the gel is twice a day until 5th day. Labial mucosa in rats was observed using Hematoxylin Eosin staining to see fibroblast cells. Data analysis used one-way Anova test and Post Hoc LSD test at  $p < 0,05$ . The results showed that the 10% bay leaf extract gel treatment group higher than the control group and the 5% bay leaf extract gel treatment group with a significant difference ( $p < 0,001$ ). The conclusion of this study is that bay leaf extract gel can increase of fibroblast cells in accelerating the ulcers healing process.

## 1. INTRODUCTION

Traumatic ulcers are the most common lesions in the oral cavity with a prevalence of 83.6% which belongs to the higher category.[1] Traumatic ulcer is described clinically as the form of lesions with yellowish fibrin exudate in the middle of the erythema edge.[2] Traumatic ulcers that occur in the oral cavity can cause pain, difficulty in doing oral activity such as chewing and patient discomfort.[3]

In general, wound healing aims to restore continuity and function of tissues in the oral mucosa which consists of 4 phases that are stacked hemostasis phase, inflammatory phase, proliferation phase, and remodeling phase.[4] The proliferation phase takes place on the 3rd to 14th day of post- traumatic stress disorder.[5] This phase is characterized by the substitution of the provisional matrix dominated by platelets and macrophages that are gradually replaced by fibroblast migration and the deposition of extracellular matrix synthesis.[6]

Fibroblasts have an important role to play in removing growth factors that will help the wound healing process.[7] Fibroblasts begin to appear in large quantities on the 3rd day after the occurrence of trauma and reach the peak levels on the 7th day.[8] The main function of fibroblasts is to maintain the integrity of support network by changing continuously the extracellular matrix.[4] Topical corticosteroids can help heal ulcers, but Indonesians remain using traditional medicine to aid healing because traditional medicine has relatively less side effects less than modern medicine.[9,10] One of the alternative ways to wound healing is to use plants for the treatment of a disease and health care.[9]

Bay leaves (*Syzygium polyantha*) are one of the plants that are often used by the community as an alternative medicine.[10] Part of bay leaf (*Syzygium polyantha*) which is usually used as a treatment is the leaves.[11] Bay leaves have polyphenol compounds, especially flavonoids, which have potential strong content as antioxidants.[12]

Flavonoid groups contained in bay leaves play a role in inhibiting the release of inflammatory mediators, as already known that bay leaves have anti-inflammatory abilities.[13] Anti-inflammatory activity on bay leaves works to inhibit the path of cyclooxygenase in the metabolic pathways of arachidonic acid.[14]

Gel bay leaf extract (*Syzygium polyanthum*) with a concentration of 10% can provide anti-inflammatory effect in the form of decreased expression of TNF- A and increased expression of VEGF in the healing process of traumatic ulcers.[15,16] The concentration of 5% of bay leaf extract (*Syzygium polyanthum*) has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. [14] The purpose of this study is to observe the effect of 5% and 10% of the bay leaf extract gel on the number of fibroblasts in the healing process of traumatic ulcers.

## 2. METHODOLOGY

This current research is an experimental laboratory with post-test only control group design. The study sample used 15 white male rats (*Rattus novergicus*) Wistar strain with the age of 2-3 months in an average weight of 200-250 grams. The criteria of healthy mice are characterized by the active movement of the mice and zero record of experimental usage. The mice were grouped into 3 and adapted for 7 days with standard drinking feeding, followed by the manufacture of ulcers using a heated 2mm diameter round burnisher tip.

Bay leaf extract used in this study is green bay leaves from the Family Medicinal Plants Kelurahan Pusponjolo, Semarang with characteristics non-dry and not brownish in color. Next, the leaves are cut into small pieces and put in the oven to dry. Dried leaves are mashed to be used as simplisia. Simplisia dissolved in alcohol 96% with a ratio of 1:10. The solution is stirred for 30 minutes and soaked for 5 days. After 5 days the solution is filtered and continued with the thickening of the liquid using a rotary evaporator (Heidolph). Thickening is continued by using a water bath.

The gel is made with 0.2% of methyl paraben in distilled water and then heated up to 70°C. The addition of Carbomer is used to help gel formation. Furthermore, the 2000mg of the bay leaf extract is required to make 20mL of bay leaf extract gel with a concentration of 10% and the 1000mg of the bay leaf extract is required to make 20mL of gel at a concentration of 5%. After the gel is formed, 10% glycerin, 5% triethanolamine and neutralizer pH triethanolamine were added. The gel was applied regularly in the morning and afternoon for 5 days using a cotton bud.

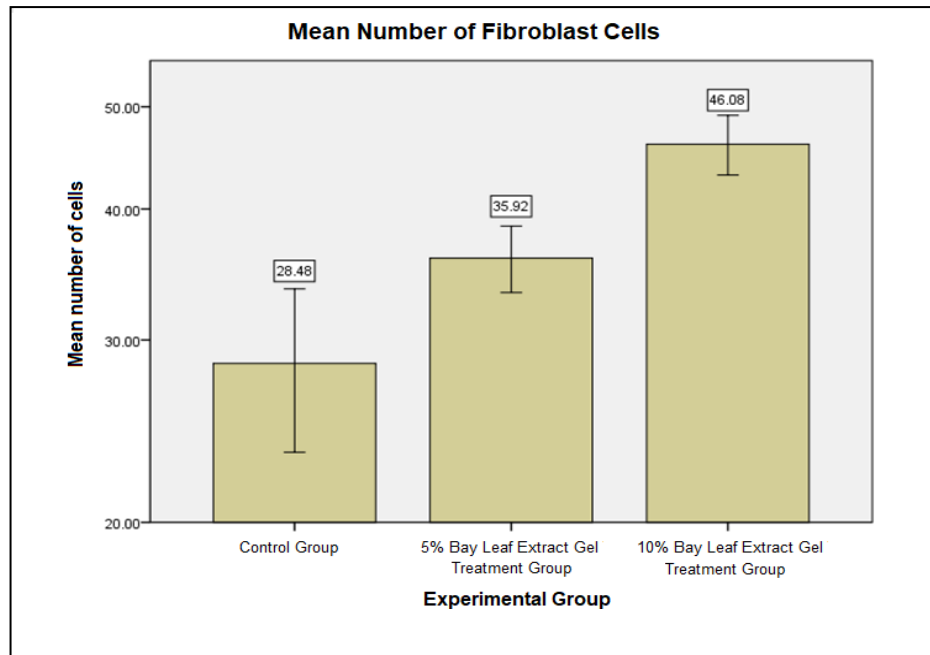
The test animals were decapitated on the 5th day and mucosal tissue was taken by cutting the labial to the corners of the mouth that have ulcers. The tissue was fixated in the 10% of formaldehyde solution and labeled. After 24 hours, the dehydration process of the tissues was carried out gradually using alcohol. After that, clearing process was done, the tissue appears transparent by inserting the tissue into the xylol solution, followed by the creation of paraffin blocks. Preparations were made by cutting paraffin blocks using a microtome, then gradually rehydrated and colored with Hematoxylin Eosin. Histopathological assessments were observed and calculated in 5 different points of view with a magnification of 400x using a light microscope.

The data were analyzed using IBM SPSS Statistics software with Shapiro- Wilk normality test (normal criteria  $p > 0.05$ ) and for variant homogeneity, the data were tested using Lavene's test. Different tests using Anova's one-way parametric statistics and continued post hoc LSD tests.

## 3. RESULTS

Data collection was conducted at the Biology Laboratory in Universitas Negeri Semarang under permit from KEPK FK Universitas Diponegoro with No.28/EC/H/FK-UNDIP/III/2021. The average results of fibroblast are seen through statistical analysis of the number of fibroblast cells in Figure 1.

Figure 1. Diagram of the average number of fibroblast cells traumatic ulcer labialmucosa of wistar rats with the standard value of errors (day 5)



Data analysis continued using Saphiro-wilk normality test and obtained normal distribution data ( $p > 0.05$ ). Homogeneity test results can be seen in the Table 1. The significance figure in all treatment groups shows  $p = 0.445$  which means  $p > 0.05$ , it can be said that the distribution of homogeneous data then the analysis was done using Anova one way test.

Group	N	Average $\pm$ SE	Test Saphiro-wilk	Lavene Test	ANOVA test
K1	5	28,48 $\pm$ 2,54	0,450		
K2	5	35,92 $\pm$ 1,31	0,754	0,445	0,000
K3	5	46,08 $\pm$ 1,50	0,516		

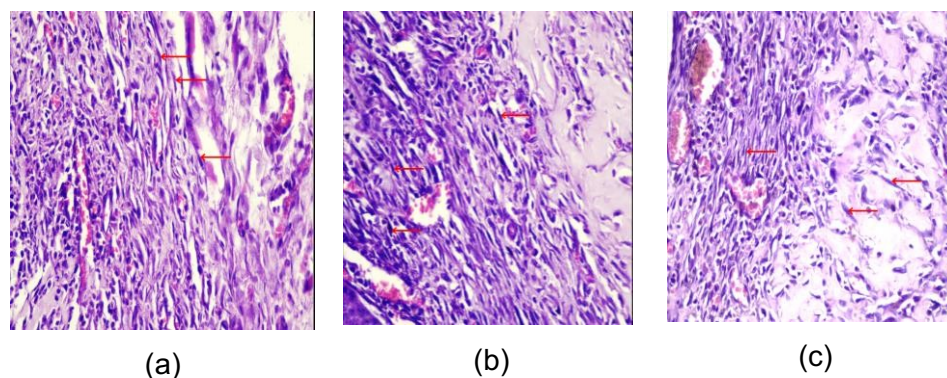
Table 1. Comparative test results of average number of fibroblast cells. K1: Negative control; K2: treatment using bay leaf extract gel 5%; K3: treatment using bay leaf extract gel 10%

One-way Anova test in Table 1 shows a value of 0.000, it can be interpreted that there is a significant influence on the number of fibroblast cells between groups. Table 2 shows that the results of post hoc LSD tests between groups have a significant value of  $p < 0.05$ , it can be said that there is a significant difference in the average number of fibroblast cells between each group. The oval, flat and blackish purple color fibroblast cells found in the glassobject of the tissues (Figure 2).

	Group	Group	Significance
LSD	K1	K2	0,015*
		K3	0,000*
	K2	K1	0,015*
		K3	0,002*
	K3	K1	0,000*
		K2	0,002*

Table 2. The results of Post Hoc LSD test.: \* Significant average difference at a rate of 0.05.

Figure 2. The results of fibroblast cells observation (red arrows) in the glass object of the tissues of labial mucosa of mice wistar. Hematoxylin Eosin Staining (400x). (a) control group; (b) group of gel extract bay leaf 5%; (c) group of bay leaf extract gel 10%



#### 4. DISCUSSION

The results show that there is an effect of bay leaf extract gel on the increase of fibroblast cells in the healing of traumatic ulcers. The ethanol extract of bay leaf is used because of its secondary metabolite content such as flavonoid, essential oils, and tannins.[17] The other compounds contained in the bay leaf extract are vitamin A, B complex, vitamin C, protein, zinc, and carbohydrates.[17,18] According to Linder in Cresna et al revealed that vitamin C has an essential role in the synthesis of collagen, thus strengthening blood vessels for wound healing. The vitamins contained in bay leaves can promote cell regeneration.[17] The effect of bay leaf extract gel on the increase in the number of fibroblast cells due to the presence of flavonoid content that is anti-inflammatory.[19] The mechanism of flavonoid as an anti-inflammatory is almost the same as the way non-steroidal anti-inflammatory drugs (AINS) work.[20] AINS acts as an anti-inflammatory by inhibiting the synthesis of prostaglandins in the cyclooxygenase pathway and inhibiting the accumulation of leukocytes in tissue damage areas.[11,19,21] Another role of flavonoid as anti-inflammatory is that it can decrease the secretion of pro-inflammatory cytokines that have analgesic properties and increase anti-inflammatory cytokines that have analgesic properties.[22]

Pro-inflammatory cytokines produce IL-1, IL-6, and TNF-A which function to regulate growth, cell activation, differentiation, and placement of cells, produced in large quantities at the beginning of the wound to increase the permeability of blood vessels.[23] The excessive pro-inflammatory cytokines will cause inhibition of angiogenesis and fibroblast cell migration which cause delayed wound healing.[16,19–25] The bay leaf extract gel treatment group in this study has an effect in increasing the number of fibroblasts compared to the negative control group. This is supported by research Savira et al showed a significant effect on the bay leaf extract gel in lowering the expression of TNF-A in the healing process of traumatic ulcers of the oral mucosa.[16] Decreased expression of TNF-A may increase fibroblast cell migration activity in the area of trauma occurrence.

Anti-inflammatory cytokines such as IL-4, IL-10 and TGF-B are produced in the advanced phase of inflammation.[25] TGF-B, TGF-A along with FGF, and VEGF play a role in initiating the proliferation phase by inducing the migration of fibroblast cells into the fibrin matrix which will be replaced by granulation tissue so that extracellular matrices will be formed.[20,26] This study obtained the results of bay leaf extract gel affects the increase of fibroblast cells in the healing of traumatic ulcers.

The results are in line with Rizal et al research which suggests that the average number of VEGF expression in the bay leaf extract gel treatment group is higher than the average control of gel carbomer. Furthermore, Rosada et al research suggests that the content of bay leaf is able to increase the number of cells expressing FGF.[15,27] The increased expression of VEGF and FGF has an effect on the number of fibroblasts migrating to the wound gap.[20]

The gel of 5% bay leaf extract in this study has an effect in the increase of fibroblast cells compared to the negative control group. This is reflected by the research Warnida et al obtained results that bay leaf extract 5% have antibacterial activity.[14] Antibacterial activity in the bay leaf extract is able to reduce the growth of inflammatory-causing bacteria.[11] However, the result is not higher than the treatment group of leaf extract gel 10% due to the increased concentration of bay leaf content that can affect the acceleration of the wound healing process.[17] This is supported by Kurniawan et al research which suggests that 10% bay leaf extract can speed up the healing process of traumatic ulcers.[15]

## 5. CONCLUSION

Based on the results of the study, it can be concluded that bay leaf extract affects the increase of fibroblast cells in the healing process of traumatic ulcers. Increased number of fibroblast cells can accelerate the proliferation process which helps wound healing becomes faster.

## Conflict of Interest

The authors declare no conflicts of interest in this study.

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Not applicable.

## Authors Contribution

Conceptualization: IAK, HI, OS; Methodology: KF, IAK, HI, OF; Formal Analysis KF, IAK, HI, OF; Data Curation KF, IAK; Original Draft Writing: KF, IAK; Supervision and Editing: IAK, HI, OF; and Administration: KF.

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