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# Effect of 5% Binahong (*Anredera cordifolia*) Leaf Extract in Lymphocyte Amount at The Tooth Extraction Wound Healing Process of Wistar Rat

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## ABSTRACT

***Introduction*:** The growth of lymphocyte cells is one indication that the wound is healing. Binahong leaves contain flavonoids, saponins, tannins, terpenoids, and oleanolic acid that influence lymphocyte activity. This study aims to analyze the effect of a 5% gel made from binahong leaf extract on the number of lymphocytes throughout the healing process of wounds post tooth extraction.

***Methods*:** This research type is pure experimental research with a posttest-only control group design with ethical approval from Dr. Moewardi Hospital Number 1.847/X/HREC/2023. The research subjects were 36 mice divided into negative, positive, and treatment control groups. The number of lymphocytes was counted using a microscope with a magnification of 1000 times.

***Results*:** The results showed a significant difference in the number of lymphocyte cells between groups ($p < 0.05$) on days 5, 7, and 14. LSD results showed a significant difference ($p < 0.05$) between the treatment group and the negative control (day 2). -5, 7, and 14) with positive control (day 5).

***Conclusion*:** Shift in the peak number of lymphocytes in the treatment group compared to the negative and positive control groups. After tooth extraction, the amount of lymphocyte cells in the wound healing process is impacted by 5% binahong leaf extract gel.

***Keywords*:** Binahong leaf extract gel, Lymphocyte, Wound healing

## Introduction

A person's quality of life will be impacted by wounds in the socket following tooth extraction, particularly if they have impaired masticatory and phonetic skills (Hanafiah et al., 2022). According to Ningsih (2018), wounds in tooth sockets will go through a wound-healing process that includes phases for proliferation, remodeling, inflammation, and homeostasis. Lymphocyte cells are one type of inflammatory cell that is present in the wound area during the inflammatory phase (Primadina et al., 2019).

The role of lymphocytes in the wound healing process is to release lymphokines which influence the speed of chemotaxis and macrophage aggregation in the wound area (Robbins & Kumar., 2013). Lymphocytes also stimulate the production of extracellular matrix and collagen, so a lack of lymphocyte cells will disrupt collagen synthesis and wound healing will not be optimal (Izzaty et al., 2014). Lymphocytes are also able to kill pathogenic microorganisms such as bacteria, fungi, and viruses (Prakoeswa, 2020) and play a role in the formation of antibodies (Short et al., 2021).

The inflammatory phase is a phase that must be passed because it is a form of protective response of the body's immune system against injury (Izzaty et al., 2014) and prevents infection in wounds (Gupta & Kumar., 2015). However, the inflammatory phase should not last too long because it will cause more severe tissue damage and hinder the wound-

healing process (Izzaty et al., 2014). Application of glycerin iodine after tooth extraction in the socket area can help the wound healing process because glycerin iodine is antiseptic and anti-inflammatory (Baroro & Utami., 2015). Glycerin iodine has side effects in the form of inflammatory reactions and inhibits the fibroblast proliferation process with long-term use (Putrianirma et al., 2019).

Binahong leaves contain several chemical compounds such as flavonoids, saponins, terpenoids, tannins, alkaloids, and oleanolic acid, which affect lymphocytes in the wound healing process (Astuti, 2019). Flavonoids are anti-inflammatory and antibacterial (Hosseinzade et al., 2019). Saponins increase the antibody response of lymphocyte cells (Sharma et al., 2020). Tannins increase lymphocyte cell regeneration, are antibacterial, and induce Vascular Endothelial Growth Factor (VEGF) (Nurrani, 2015). Terpenoids and oleanolic acid are immunomodulatory and antibacterial (Khan et al., 2021).

In another previous study, Mutiara et al. (2015) discovered that the best concentration of binahong leaf extract gel for wound treatment following tooth extraction is 5%. It has been demonstrated that applying a 5% concentration of gel containing binahong leaf extract speeds up the healing process in Wistar rats' post-tooth extraction wounds by lowering the macrophage cell count. Binahong leaves contain active chemicals that speed up the healing process of wounds by inducing macrophage formation during the inflammatory phase. According to Mutiara et al. (2015), saponins, flavonoids, tannins, and alkaloids have antimicrobial qualities that speed up the healing process of wounds and reduce the number of macrophages produced during the proliferation phase.

According to the description given above, the researchers want to know how 5% binahong leaf extract gel affects lymphocytes, which are inflammatory cells involved in the healing process of wounds after tooth extraction. The purpose of this study is to ascertain how much of a 5% gel made from binahong leaf extract affects the number of lymphocyte cells involved in the healing of wounds following tooth extraction in Wistar rats.

## Method

This type of research is pure laboratory experimental research with a posttest-only control group design. This research was carried out at the Integrated Research and Testing Laboratory Unit IV at Gadjah Mada University (LPPT-UGM Unit IV) after an ethical commission test at the Moewardi Surakarta Regional Hospital Number 1.847/X/HREC/2023. The research material used was binahong leaves (*Anredera cordifolia*) which had previously been determined at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Muhammadiyah University, Surakarta.

Binahong leaves are made into powder and then macerated by soaking in 2 liters of 70% ethanol for 5 days, stirring occasionally with a spatula. The results of maceration with 70% ethanol solvent, and binahong leaf extract were mixed with 1% CMC-Na to make a gel preparation. The research subjects were 36 male Wistar strain white rats (Rattus novergicus), two months of age, and a body weight of 200–250 grams. The rats were split into three groups: the treatment group, the positive control group, and the negative control group.

Before having their teeth extracted, the experimental animals were given an intramuscular dose of ketamine (50 mg/kg BW) in the upper thigh. Rats' left central incisors were extracted from their lower jaws using tweezers and a needle holder. The treatment group received 5% binahong leaf extract gel, the positive control group received glycerin iodine, and the negative control group received CMC-Na 1%. After removal, the application is completed and left for ten minutes. After the teeth were extracted, the rats were killed on days 3, 5, 7, and 14. Their jaws were then cut open to prepare the tissues for histology using hematoxylin and eosin staining.

Using a light microscope with 1000x magnification in 5 distinct fields of view, the number of lymphocyte cells in the preparations was counted. One-way ANOVA and Post Hoc LSD (Least Significance Different) tests are utilized in this parametric test.


Figure 1. (1) First visual field area; (2) Second visual field area; (3) Third visual field area; (4) Fourth visual field area; (5) Fifth visual field area; (The Wistar rat mandibular preparate)

## Result and Discussion

### *Result*

The image of the lymphocytes obtained was round, purplish blue colored cells as in Figure 2. The results of the normality and homogeneity tests showed that the data were normally and homogeneously distributed, then a parametric test was carried out using the One-way ANOVA test. The results of the One-way ANOVA test showed that on the 5th, 7th, and 14th days, the p-value was <0.05, which means there was a difference in the average number of lymphocyte cells on these three days. Post hoc test results are presented in Table 1.

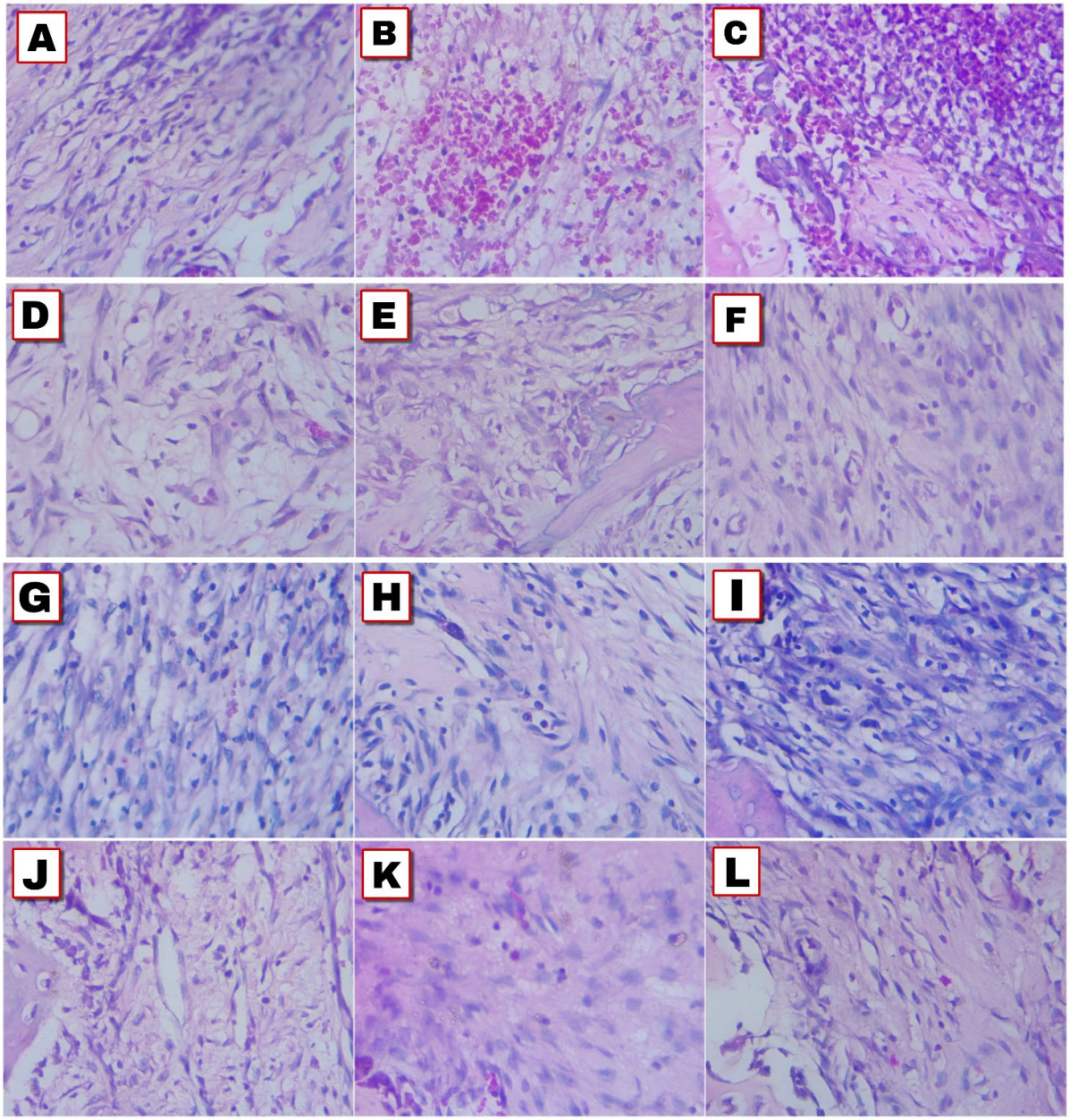


Figure 2. (A) Treatment on day 3, (B) Negative control on day 3, (C) Positive control on day 3; (D) Treatment day 5, (E) Negative control day 5, (F) Positive control day 5; (G) Treatment day 7, (H) Negative control day 7, (I) Positive control day 7; (J) Treatment day 14, (K) Negative control day 14 (L) Positive control day 14; (400x magnification)

Table 1. Summary of LSD analysis of lymphocyte cells between treatment groups on various observation days

| Group | Days | *p-value* |
|---|---|---|
| Negative Control-Treatment | 5 | 0,002* |
| | 7 | 0,004* |
| | 14 | 0,011* |
| Positive Control-Treatment | 5 | 0,000* |
| | 7 | 0,139 |
| | 14 | 0,499 |

Information: * (p-value < 0.05): there is a significant difference

The average results and standard deviation of calculating the amount of lymphocyte cells involved in the healing process of the wound following the extraction of a rat tooth are presented in Figure 3. The positive control and negative control groups both have a graphic pattern like a horse's saddle, namely the highest average number of lymphocytes is on the observation 3rd day, then decreased on the 5th day of observation. There was an increase again on the 7th day of observation, but on the 14th day of observation, there was a decrease. Meanwhile, on the fifth day of observation, the therapy group had the greatest average number of lymphocytes, up from the third day of observation. On the seventh and fourteenth days of observation, this number also decreased.

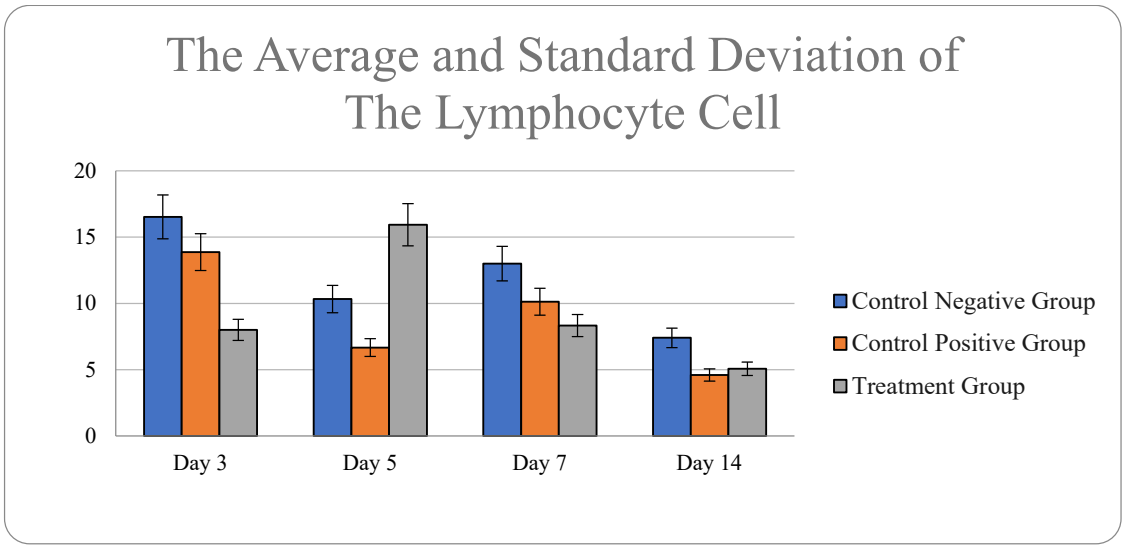


Figure 3. Graph of the average and standard deviation of lymphocyte cells

### *Discussion*

The treatment group had fewer lymphocyte cells on day three following damage than both the negative and positive control groups. This is because, in the treatment group, active compounds from binahong leaves, such as flavonoids and oleanolic acid, which were able to inhibit the inflammatory process in the arachidonic acid pathway and saponin compounds, which produce anti-inflammatory cytokines, prevented the acute inflammatory process from occurring on day three (Mutiara *et al*., 2015), so that the formation of the number of lymphocyte cells becomes smaller. Acute inflammation in post-extraction wounds can be caused by microflora such as bacteria in the oral cavity (Gowda *et al*., 2013).

Bacteria release endotoxins which will then be recognized by receptors on lymphocyte cells, then lymphocytes will eradicate bacteria from the wound area through the endocytic pathway (Prakoeswa, 2020). The binahong plant has antibacterial properties, this is in line with the previous study by Mengga et al. (2022) binahong leaf extract effectively inhibits the growth of *Staphylococcus aureus* bacteria at concentrations of 5%, 10%, and 15%. Other previous research by Helmi et al. (2022), binahong leaf extract is also able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*

bacteria. The *Staphylococcus* family of bacteria are the main microorganisms that inhabit our skin and mucous membranes. If there is a wound, *Staphylococcus* is one of the bacteria that colonize into a biofilm, thereby disrupting the wound healing process (Halim et al., 2022). The flavonoid compounds contained in binahong leaves have been proven for their antibacterial properties due to their accessibility and safe therapeutic use (Zulkefli et al., 2023). Apart from that, there are also saponin, tannin, and alkaloid compounds which are also antibacterial (Hanafiah et al., 2022).

The control group's lymphocyte count dropped while the treatment group's grew on the fifth day following injury. The LSD test revealed that there was a significant difference in the number of lymphocytes in the treatment and control groups, with the p-value between the treatment groups with positive control and negative control being less than 0.05. This demonstrates that compared to Na-CMC and glycerin iod, binahong leaf extract gel has a greater capacity to stimulate lymphocyte cells arriving at the wound site. Increased phagocytic activity against germs and dead cellular debris was linked to the increase in lymphocyte cells on day 5. Previous research by Ariami et al. (2021), binahong leaf extract is proven to be immunostimulatory, characterized by an increase in Widal titer induced by *Salmonella typhi* antigen, the flavonoid and alkaloid content in binahong leaf extract is immunostimulatory by increasing the secretion of IL-2 (Interleukin 2) thereby increasing the proliferation and differentiation of lymphocyte cells as well as increased antibodies. An increase in the number of lymphocytes will also affect the production of macrophage cells. This is due to lymphocytes producing lymphokine that stimulate macrophage production (Guyton & Hall., 2020). The binahong leaves contain flavonoid compounds that can stimulate lymphocytes to produce the cytokine IFN-γ (Interferon-γ) which is the main cytokine of MAC (Macrophage Activating Cytokine) to activate macrophages and increase phagocytic activity (Robbins & Kumar., 2013).

On day 7 saw a decrease in the number of lymphocyte cells in the treatment group and an increase in both the positive and negative control groups.The decrease in lymphocyte cells in the treatment group indicates that the antigen has disappeared and the formation of fibroblast cells in the wound area. Previous research by Ratu et al. (2019) binahong leaf extract has been proven to increase the number of fibroblasts and collagen thickness. Saponin compounds from binahong leaves will increase fibroblast growth factor (FGF) and IL-2 (Interleukin 2) so that fibroblast cells and epithelial cells migrate to the wound matrix (Hertian et al., 2021). Lymphocytes suppress the proinflammatory cytokine IFN-γ (Interferon γ) which results in the change of M1 phenotype macrophages into M2 phenotype macrophages (Das et al., 2015). Changes in macrophage phenotype from M1 to M2 will release IL-10 (Interleukin 10), inhibit the production of IL-1β (Interleukin 1β) and TNF-α (Tumor Necrosis Factor α), and the inflammatory process will soon pass and continue to the next process, namely the proliferation and remodeling phase (Das et al., 2015; Rehak et al., 2022). Lack of lymphocyte cells will interfere with collagen synthesis and wound healing will be slow (Izzaty et al., 2014).

The number of lymphocytes that rose again in the negative and positive control groups was related to phagocytic activity which had not been maximal on the previous day. Lymphocyte cells not only play a role in eliminating extrinsic factors that cause inflammation such as bacteria, fungi, and viruses but also play a role in assisting the phagocytic activity of macrophages in eliminating intrinsic factors that cause inflammation such as dead cell debris in the wound area (Papenfuss & Bolon., 2014). Day 5 marked the treatment group's greatest production of lymphocyte cells, indicating that phagocytic activity maximized on that day. As a result, the next day showed a decrease in the amount of lymphocyte cells due to the removal of the inflammatory substances. On day 7, the number of lymphocyte cells increased in both the positive and negative control groups, indicating that the inflammatory process continued to go on. This could have been brought on by bacteria and necrotic cell debris in the wound area that had not been completely phagocytosed the day previously. Flavonoids, saponins, terpenoids and alkaloids are able to stimulate pro-inflammatory cytokines, thereby producing more inflammatory cells in the wound area, namely lymphocytes and natural killer cells (Zulkarnain et al., 2020).

Day 14 revealed a drop in the amount of lymphocyte cells across all groups, indicating the completion of the inflammatory phase and the beginning of the phase of proliferation. The proliferative phase involves keratinocytes, fibroblasts, M2 phenotype macrophages, Treg lymphocytes, and endothelial cells, which work together to promote re-epithelialization, angiogenesis, and fibroplasia (Wilkinson & Hardman., 2020). Previous research by Septiana et al. (2019), proved that binahong leaf gel extract was able to increase wound re-epithelialization after tooth extraction in male Wistar rats. Binahong leaves contain several compounds that influence the re-epithelialization process, like like oleanolic acid, flavonoids, alkaloids, saponins, tannins, and ascorbic acid. Flavonoid compounds, saponins, and oleanolic acid are able to activate the growth factor, namely TGF-β (Transforming Growth Factor β), which increases the proliferation and migration process of epithelial cells to the wound area (Ardiana et al., 2015). Tannin and alkaloid compounds also have antioxidant properties which will protect cell membranes from free radicals and pathogenic bacteria (Hanafiah et al., 2017). Tannin compounds will influence the proliferation, differentiation and migration of epithelial cells to the wound area so that the epithelial cells immediately close and become thicker (Sa'diyah et al., 2019). Ascorbic acid contained in binahong leaves have functions as an antioxidant. Ascorbic acid also serves as a cofactor in the synthesis of collagen and proteoglycans (Ningsih et al., 2019). It can enhance the integrity and strength of the wound in the re-epithelialization process (Kartiningtyas et al., 2015).

According to the results of phytochemical tests carried out by Astuti (2019), Binahong leaves contain chemical compounds such as oleanolic acid, ascorbic acid, terpenoids, alkaloids, flavonoids, and steroids which are useful for wound healing process. The flavonoids contained in binahong leaves are anti-inflammatory because they can inhibit pro-inflammatory cytokines and inhibit serine-threonine protein kinase binding in the arachidonic acid metabolism pathway

(Sangeetha et al., 2016). Alkaloids are immunomodulatory and have antibacterial properties by inhibiting the adhesion of bacterial proteins to polysaccharide receptors (Tjahjani & Yusniawati., 2017; Khan et al., 2021). Terpenoids and steroids are anti-inflammatory, antibacterial, and able to promote fibroblast formation (Sari et al., 2023). Saponins increase antibody responses and are anti-inflammatory and antibacterial (Izzati, 2015; Rahmawatiani et al., 2020). Oleanolic acid is also known to increase the activity of cicatrizant or can reduce pain and increase the rate of activity of the wound healing process (Akhmadi et al., 2022).

Delivering 5% gel derived from binahong leaf extract can progressively lower the daily count of lymphocytes. An inflammatory cell type called lymphocytes is involved in the wound-healing process following tooth extraction (Short et al., 2021).. A decrease in tissue infection is indicated by a decrease in lymphocyte cells between the treatment and control groups. Day 5 marks the highest shift in lymphocyte production, which then decreases on Days 7 and 14, indicating that the inflammatory process is short-term and assists in the faster healing of wounds.

This study's limitation is that the 5% binahong leaf extract gel hasn't undergone a biocompatibility test. In order to do more research, the author recommends testing the biocompatibility of a gel containing 5% binahong leaf extract on the healing process of wounds following tooth extraction.

## Conclusion

The amount of lymphocyte cells in the wound healing process post tooth extraction in white rats (*Rattus novergicus)* Wistar strain was affected by the administration of binahong (Anredera cordifolia) leaf extract gel at a concentration of 5%. After tooth extraction, the peak shift in lymphocyte cell production during therapy happens faster, accelerating the healing of the wound.

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