The Effect of Ginger Extract (Zingiber officinale Roscoe) on the Number of Neutrophil Cells, Fibroblast and Epithelialization on Incision Wound

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Abstract - The wound healing process consists of three phases, namely the inflammatory phase, proliferation, and maturation. An increase in the number of neutrophil cells and macrophages signifies an inflammatory phase. Fibroblasts and epithelialization indicate a proliferation phase, whereas in the maturation phase is marked by the occurrence of wound healing. Ginger (Zingiber officinale Roscoe) contains active substances, namely triterpenoids, flavonoids and saponins. Flavonoids function as anti-inflammatory. Although it has many benefits, the effect of giving ginger extract to neutrophil cells, fibroblasts, and epithelial thickness in incision wounds has not been studied. The purpose of this study was to determine the effect of ginger extract on the number of neutrophil, fibroblast cells, and epithelialization in incision wounds. The research design used was post test only control group design. Rats were divided into control groups given 1% CMC solvents and the treatment group were given oral ginger extract at a dose of 1 g / kg BW. The tissue was observed on days 1, 5 and 10. Based on the results of the Kruskall Wallis test, the p value was 0,000 with 0,05, so it can be concluded that the administration of ginger (Zingiber officinale Roscoe) can reduce the number of neutrophil cells, increase the number of fibroblast cells, and increase epithelialization of incision wounds in white mice (Rattus norvegicus).

Keywords: ginger extract, fibroblasts, neutrophil cells, epithelialized incisional wounds

1. Introduction
Wounds are discontinuities of a network [1]. Wound incision is made with a clean cut using a sharp instrument [2]. Wound healing through three main phases, namely the inflammatory phase, proliferation, and maturation [3]. Neutrophil cell activity and macrophages indicate an inflammatory phase. The proliferation phase is characterized by the presence of fibroblasts and epithelialization, then the maturation phase is characterized by wound healing [4]. The time required for each phase is different, if the wound treatment is good, the wound will heal quickly, but if complications occur it will prolong wound healing.

So that the wound heals faster, proper wound care is needed and accompanied by antibiotic use. Today's herbal plants are increasingly in demand as an alternative therapy that is not less important than medical therapy and has mild side effects [5]. One type of herbal plant that is often found in Indonesia is ginger (Zingiber officinale Roscoe). Ginger has active substances namely oleoresin, gingerol, shogaol and flavonoids. Gingerol and shogaol are
phenolic components of ginger which are known to have anti-inflammatory effects, anti-cancer, and antitumor [6]–[8]. Although it has a lot of active ingredients, the effect of giving ginger extract to neutrophil cells, fibroblasts, and epithelial thickness in incision wounds has not been studied [9].

One study of ginger is a research which examined ethanolic extract of ginger which has an anti-inflammatory effects in mice. Ethanol extract of the large ginger rhizome (Zingiber officinale Roscoe) given orally at a dose of 30, 100, 300 mg/kg body weight [10]. Based on the research, the dose used in this study was 1 g / kg body weight. Because of the high benefits of ginger, experimental research on ginger specifically regarding the effects of ginger extract (Zingiber officinale Roscoe) on the number of neutrophil cells, fibroblasts, and thick epithelialization of incision wounds in white rats (Rattus norvegicus) is needed.

2. Method
2.1 Sample
Male white rats (Rattus norvegicus) obtained from the biochemistry laboratory of FK Airlangga University. Mice used were 2 months old with a weight of 200 grams ± 10%. White rats were acclimatized for a week, kept in cages of size 40 x 30 cm which were covered by wire mesh and equipped with a place to eat and drink and lay on husks, one cage was occupied by one mouse. White mouse foods use standard food and drink bottled water. Food is given with adlibitum. To maintain the cleanliness of the cage, the husk is changed every 2 days.

2.2 Materials and research tools
The materials used in this research are wound making materials including 70% alcohol, distilled water, and local anesthetic drugs (Lidocaine), wound care materials namely physiological solutions, examination materials include white mouse skin tissue, materials for making histological preparations paraffin method consisting of Bouin's solution for fixation (made from saturated picric acid 1.22% by 750mL, Formaldehyde 37-40% by 250 mL, glacial acetic acid by 50 mL, alcohol 70%, 80%, 90%, 95% and absolute for dehydration, Xylo or Xylene solution for clearing, liquid Paraffin for tissue blocks, Meyyer albumin made from egg white and glycerin 1:1, and enthelan for mounting), material for Hematoxyline Eosin staining (consisting of Xylol solution, absolute alcohol, tap water, Hematoxyline solution, 1% acid alcohol, ammonia solution, and eosin solution). For ginger extract obtained from the Faculty of Pharmacy, Airlangga University.

The tools used in this study are tools for the maintenance of rats including maintenance cages, bottles for drinking places, food containers, tools for making incisions including razors and handles, scalpels, rulers and markers, cotton, sterile gauze, perlak, clean gloves, plaster, scissors, 3 ml syringe, and crooked, wound care equipment includes sterile wound care sets, sterile gloves, wound dressing, bent, perlak, plaster, cotton, scissors, and com, a tool for the administration of oral extracts namely sonde, tool for anesthesia and extraction of skin tissue including 1 ml syringes, experimental fixation and desection devices, small bottles with lids for tissue fixation, and a set of minor surgical instruments, tools for making histological preparations.
include microtomes, glass objects and glass covers, metal molds L-shaped for embedding, water bath, staining jar, light microscope, and micrometer measuring device.

2.3 Research design This research is a true experiment.

2.4 Variable
The independent variable in this study was ginger extract while the dependent variable in this study was neutrophils, proliferation, and epithelialization

2.5 Research procedure
Rats were randomized to the control and treatment groups. the control group was given 1% CMC solvent and the treatment group was given 1gr /kg ginger extract. The control and treatment groups were further divided into control group 1 and treatment 1 (tissue taken first day), control group 5 and treatment 5 (tissue taken fifth day), control group 10 and treatment 10 (tissue taken tenth day). At the time of excision of the wound, the tissue around the wound was also removed approximately 0.5 cm. The wound tissue samples after excision were placed and wrapped using filter paper which was given a hole and then fixed by placing 10% formalin for 4-5 days, then histological preparations were made. Next was the observation of histological preparations of wound tissue that had been made into slides under a light microscope.

2.6 Data analysis
The data were analyzed by the Kruskall wallis test then followed by the Mann-Whitney U test.

3. Result and Discussion
3.1 Descriptive analysis

![Graph of mean control group neutrophils and treatment cells](image)

Figure 1 Graph of mean control group neutrophils and treatment cells

Based on Figure 1 it can be seen that in the control and treatment groups, both experienced a decrease in the number of neutrophil cells, but the treatment group had fewer neutrophil cells compared to the control group.
Figure 2 Graph of mean control and treatment fibroblast cell groups

From Figure 2 it can be seen that in the two groups from the first day to the fifth day there was an increase in the number of fibroblast cells, then decreased to the tenth day.

Figure 3 Graph of the mean epithelialization of the control and treatment groups

In figure 3 it can be seen that on the first day in both groups epithelialization was not found, but on the fifth day the epithelialization process increased dramatically in both groups, especially the treatment group. On the tenth day there was still an increase in epithelialization in both groups except that the increase was not as fast as on day 5.

3.2 Kruskall-Wallis Test Results

The Kruskall-Wallis test results can be seen in table 1 below:

| Variable          | N  | df | p       |
|-------------------|----|----|---------|
| Neutrophil cells  | 30 | 5  | 0.000 * |
| Fibroblast cells  | 30 | 5  | 0.000 * |
| Epithelialization | 30 | 5  | 0.000 * |

Table 1 shows that there are significant differences (p <0.05) in the neutrophil, fibroblast and epithelial cell variables with a value of p = 0,000 between the control and treatment groups. Based on the results of the Kruskall-wallis test showed that there were significant differences (p <0.05) in the neutrophil cell variable with a value of p = 0,000 between the control groups and the treatment. Initially, the highest number of neutrophils is because it is the largest fraction of peripheral white blood cells. Neutrophils cleanse the inflammatory area of infectious and toxic agents and tissue debris through phagocytic and non-phagocytic mechanisms. The presence of neutrophil activity in the wound healing process indicates that currently the wound healing process is in the inflammatory phase [3].

Ginger has active substances namely oleoresin, gingerol, shogaol and flavonoids. Gingerol and shogaol are phenolic components of ginger which are known to have anti-inflammatory effects anti-cancer and antitumor [9]. This active substance will inhibit cycloxygenase activity so that the amount of prostaglandin as an inflammatory mediator will decrease. This decrease in inflammatory mediators will reduce the number of neutrophil cells.
The inflammatory process continues within 24 - 48 hours after the wound, monocytes replace neutrophils and become the main leukocytes. Monocytes are drawn to the site of the wound by kalikrein, fibrinopeptide, and fibrin degradation products. Furthermore, monocyte deposition is taken over by specific chemoattractants, namely collagen, fibronectin, elastin, and TGF-β fragments. Monocytes undergo phenotypic changes into tissue macrophages. Macrophages phagocytosis and kill bacteria and scavenge debris tissue. These factors stimulate the migration and proliferation of fibroblasts, as well as the production and modulation of the extracellular matrix. Macrophages are the main cells and are important for wound repair. Within 1-2 days after injury, the remaining neutrophils are phagocytes by macrophages so that the number of neutrophil cells decreases. The first phase of wound healing ends, while the proliferation and tissue formation phases have been and are ongoing [12].

Based on Figure 2 it can be seen that in the two groups from the first day to the fifth day there was an increase in the number of fibroblast cells, then on the fifth day to the tenth day there was a decrease in fibroblast cells. Although in the two groups had the same tendency, the average number of fibroblast cells in the treatment group was higher when compared to the control group. This is in accordance with the mechanism of wound healing, namely in the proliferation phase characterized by increased fibroblasts [12].

Ginger flavonoids also play a role in activating macrophages [13]. As mentioned above, macrophages release several growth factors, including PDGF, fibroblast growth factor (FGF), epidermal growth factor (EGF), TGF-β, and TGF-β. These factors stimulate the migration and proliferation of fibroblasts, as well as the production and modulation of extracellular matrices [12]. This is in accordance with the results of the Kruskal-Wallis test showing that there were significant differences (p <0.05) in the fibroblast variable with a value of p = 0.000 between the control and treatment groups.

Fibroblasts begin to migrate into the wound, 48 hours after the wound occurs. Fibroblasts move along the fibroblast matrix - fibronectin which settles in early clots, and produces fibronectin which facilitates its movement. Other components of the extracellular matrix, such as tenascin, are additional signals for adhesion and movement of fibroblasts. Fibroblasts produce extracellular matrix components, including type I and III collagen, elastin, glycosaminoglycans, and proteoglycans. Type III collagen is the dominant type of collagen during early wound repair. Synthesis of type III collagen reaches a maximum of 5-7 days after injury. TGF-β stimulates fibroblasts to produce collagen types I and III. Because new connective tissue is formed, fibroblasts undergo phenotypic changes to miofibroblasts which contain a lot of actin. Miofibroblasts have a characteristic picture of fibroblasts and smooth muscle cells, and contain a lot of rough endoplasmic reticulum tissue needed to produce a large number of matrix proteins. Miofibroblasts play a major role in wound contractions and are predominant in granulation tissue. Exposure to a number of mediators, including angiotensin, prostaglandin, bradykinin, and endothelin, results in contraction of miofibroblasts [12].

On the tenth day, both groups experienced a decrease in the number of fibroblast cells. This is due to a decrease in fibroblast cell proliferation, but fibroblasts become more progressive in synthesizing collagen and fibronectin thereby increasing the number of extracellular matrices that are reduced during inflammation [14]. This decrease in the number of fibroblasts is due to the fact that some fibroblasts will undergo phenotypic changes to miofibroblasts.
Fibroblasts also play a role in stimulating keratinocyte proliferation. Keratinocyte migration plays an important role in re-coating the epidermal defect (the process of reepithelialization). In this event changes in the form of keratinocytes, cyclo-skeleton rearrangement, and expression of keratin and proteases. Changes in the keratinocyte phenotype allow migration both from the wound edge and from each adnexal structure that is still present at the base of the wound. One - two days after the wound, epidermal cells at the edge of the wound and inside the wound begin to divide and proliferate so as to increase the population of migrating cells. TGF-β is a potent inhibitor of keratinocyte proliferation, but can increase keratinocyte migration [15].

Based on Figure 3 it can be seen that on the first day in both groups epithelialization was not found, but on the fifth day the epithelialization process increased dramatically in both groups, especially the treatment group. On the tenth day there was an increase in epithelialization in both groups except that the increase was not as fast as on day 5. From this picture it was also known that epithelialization in the treatment group was thicker when compared to the control group. Based on the results of the Kruskall-Wallis test it can be concluded that there are significant differences in epithelial thickness in the treatment group and the control group with a p value of 0.000.

On the 10th day there was a decrease in the speed of epithelialization. The decline occurred in both groups because of the remodeling process needed to respond to downregulation and return to conditions that were close to such as before the wound. Mechanisms of apoptosis and enzymatic activity Matrix-degrading Metallo Proteinases (MMP) and other proteins work to get a balance in the reepithelization of new wounds[16].

4. Conclusion
1. Ginger extract (Zingiber officinale Roscoe) can reduce the number of neutrophil cells in the proliferation phase and maturation phase in rats (Rattus norvegicus) with incisional wounds
2. Ginger extract (Zingiber officinale Roscoe) can increase the number of fibroblast cells in the proliferation phase in rats (Rattus norvegicus) with incision wounds
3. Ginger extract (Zingiber officinale Roscoe) can increase epithelialization in the proliferation phase in rats (Rattus norvegicus) with incisional wounds

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