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RESEARCH ARTICLE OPEN ACCESS

ANGIOGENESIS STIMULATION IN BACTERIA-INFECTED ACUTE WOUND HEALING WITH HONEY TREATMENT IN BALB/C MICE

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ABSTRACT

The use of honey for wound healing has been carried out several times, but until now no one has compared the effectiveness of honey from various types of honey found in Indonesia for stimulating the formation of new blood vessels (*Angiogenesis*) in acute wounds infected with bacteria. The other side of wound healing that will be observed in this study is that wounds are often exacerbated by conditions of infection due to bacteria. The purpose of this study was to determine the level of stimulation of wound healing by treating honey in acute wounds infected with bacteria. This study was divided into 4 groups, namely the control group (K-MD), the first treatment group with Javanese Honey (MD-JW), the second treatment group with Kalimantan Forest Honey (MD-KLM) and the third treatment group with NTT Honey. (MD-NTT). In the results of the study, the highest VEGF levels were found in the MD-NTT group, followed by the MD-JW group, then the MD-KLM group and the lowest were in the K-MD group. The One Way Anova test obtained p>0.05 and the Post Hoc LSD test showed that the average VEGF levels in the MD-NTT, MD-JW and MD-KLM groups were significantly higher than the K-MD group (p <0.05). In this study, it can be concluded that giving honey from NTT can increase *Vascular Endothelial Growth Factor* (VEGF) levels better than honey from Java and Kalimantan in healing acute wounds infected with bacteria in BABL/C mice.

I. INTRODUCTION

Wound healing is a physiological process that can occur naturally and dynamically in the body. The wound healing process includes 4 stages; hemostasis, inflammation, proliferation (granulation, contraction, and epithelialization), and remodeling [1]. Factors that determine success in the wound healing process are the adequacy of oxygen and nutrients that are needed by cells that play a role in the wound healing process. Availability of oxygen and nutrients is determined by the presence of vascular. In the wound process, some of the vessels are damaged, which is why it is necessary to form new vascularization or what is called neovascular [2].

Angiogenesis is the process of forming new blood vessels that already exist. This process occurs the growth of new capillaries that are connected to each other to form blood vessels that are consistent or fixed in the tissue with the wound. Cytokines and growth factors involved in this process of angiogenesis are basic *Fibroblast Growth Factor* (bFGF), Transforming Growth Factor (TGF-α, TGF-β), *Vascular Endothelial Growth Factor* (VEGF) and prostaglandins [2]. The surface of endothelial cells has growth factor receptors that play an active role in dissolving the extracellular matrix to facilitate the subsequent migration and proliferation of endothelial cells [3].

Honey is thought to be a good alternative in wound care because of its ability to be anti-bacterial, anti-inflammatory and immunostimulatory [4]. This ability is inseparable from hygroscopicity, sugar content and also contains hydrogen peroxide [5]. Previous studies stated that honey has a debridement effect for wounds [6], anti-bacterial activity [7], and the ability to reduce inflammation (Ghaderi et al, 2010) and wound areas [8]. Honey's

activity in wound healing is also influenced by the characteristics of the honey itself. This difference in activity can be caused by the species of honey-producing bees/wasps, geographical location, surrounding vegetation, and possibly also due to the process of making and storing it [9]. The extent and variety of vegetation in Indonesia is very likely to cause differences in the effectiveness of honey. This research will specifically use three types of honey in Indonesia, namely Central Java Forest Honey, Kalimantan Forest Honey, Flores NTT Forest Honey. Although there may be differences in its content, honey itself is generally composed of 40% glucose, 40% fructose, 20% water, amino acids, vitamins biotin, folic acid, pyridine, thiamine, the enzyme diastase invertase, glucose oxidase, catalase, minerals [10].

The results of this study will find out how fast each type of honey will help stimulate the formation of new blood vessels (Angiogenesis) in acute bacterial-infected wounds when compared to dressing treatment and no treatment, if it shows better ability than the comparison then it can be used to treat wounds that are more cost-effective for society and Health institutions.

II. MATERIALS AND METHODS

This research is an experimental study regarding the comparison of administration of three types of natural Indonesian honey to efforts to accelerate wound healing at VEGF levels. The research was carried out at the Laboratory of Sultan Agung University Semarang in June 2023 and has passed ethical eligibility from the Semarang Ministry of Healh Polytechnic with number 0479/EA/KEPK/2023.

The tools are Surgical tools, Bactec, Individual mouse cages, Gauze, Hydrocolloid dressing, plaster, 4 mm biopsy punch, micropipette, microtube, microtome, polypropylene sheet, 2.5 mL syringe, cotton swab sterile, computer/laptop. The Reagents are three types of honey, ketamine-xylazine, VEGF kit and NaCl.

There were 24 mice used in this study which were divided into four groups. All research samples were made by making 2 wounds on the skin of the back and given a suspension of 20 µl of S. aureus bacteria. The control group was treated for wounds by bandaging (K-MD), the first treatment group was treated for wounds by administering Central Java Forest Honey and bandages (MD-JW), the second treatment group was treated for wounds by administering Kalimantan Forest Honey (MD-KLM). and bandages, and the third treatment group was treated for wounds by administering Flores NTT Forest Honey and bandages (MD-NTT). All samples in the study group were treated for wounds every day, macroscopic wound condition monitoring was carried out for seven days and observation of infection and examination of VEGF levels was carried out on the 7th day.

The data collection carried out was primary data collection which was collected directly by the researchers by directly examining the blood specimen of the mice and the data was processed using SPSS version 26.0 to be tested One-Way Anova and Post Hoc LSD with value of significance is 95%.

III. RESULTS AND DISCUSSIONS

III.1 THE HONEY ORIGIN

In this study, honey was collected from three islands in Indonesia, namely Java, Kalimantan and East Nusa Tenggara (fig. 1). Each honey in each region has different characteristics. This is due to different weather, topography and different vegetation.

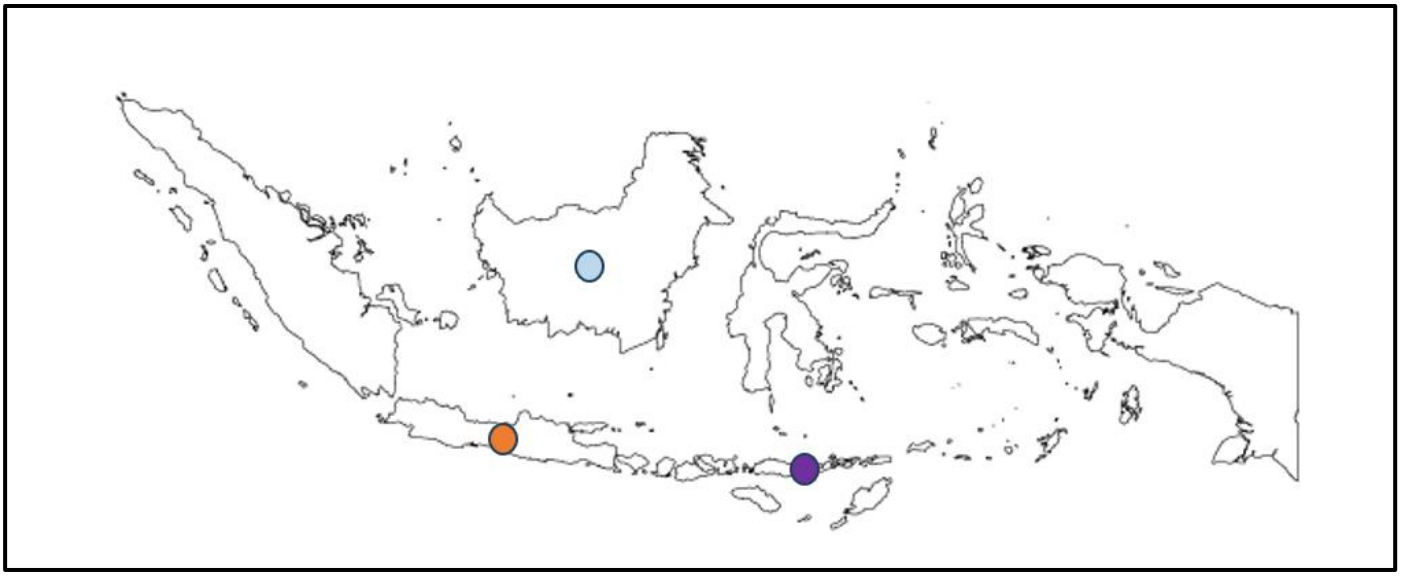

Figure 1: Map of Indonesia which consists of various islands and the origin of honey. The red color indicates the island of Java, the blue color is the island of Kalimantan, the purple color is the island of East Nusa Tenggara.
Source: Authors, (2023).

III.2 THE VEGF LEVELS

This study used 24 mice divided into 4 groups. The control group (K-MD), the first treatment group was treated for wounds by administering Central Java Forest Honey and bandages (MD-JW), the second treatment group was treated for wounds by administering Kalimantan Forest Honey (MD-KLM) and bandages, and the treatment group the third was wound care by administering Flores NTT Forest Honey and bandages (MD-NTT) then continued with blood sampling for examination of VEGF levels as a marker of angiogenesis. The results of VEGF levels are as shown in Table 1.

Table 1: Mean Results of *VEGF* levels.

Variable	K-MD	MD-JW	MD-KLM	MD-NTT	P values (Anova)
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	
***VEGF* levels**	68.24±19.94	103.49±27.65	94.32±25.19	118.7±29.01	0.043
Shapiro-Wilk	0.842	0.643	0.523	0.763	
Levene Test	0.739				

Source: Authors, (2023).

Based on Table 1, it can be seen that the highest VEGF levels were found in the MD-NTT group, followed by the MD-JW group, then the MD-KLM group and the lowest was in the K-MD group. The normality test was carried out using the Shapiro Wilk and the homogeneity test using the Levene test showed that the data in this study were normally distributed and homogeneous (p> 0.05).

Based on these results, it was continued with the *One Way Anova* test and it turned out that there was a significant difference between groups, p <0.05. To find out which group had a significant difference, an LSD Post Hoc test was carried out. These results are explained as follows:

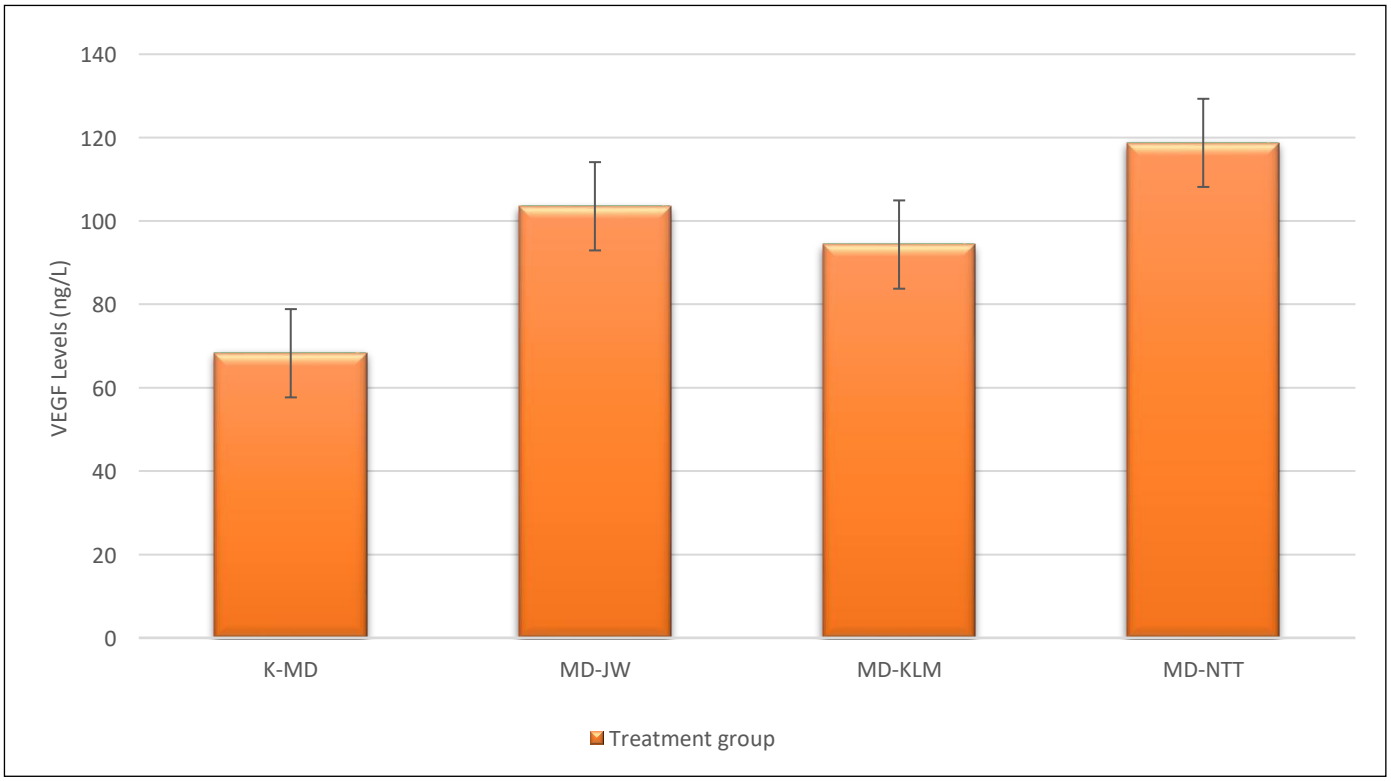

Figure 2: VEGF Levels.
Source: Authors, (2023).

The results of the LSD Post Hoc test showed that the average VEGF level in the MD-NTT, MD-JW and MD-KLM groups was significantly higher than the K-MD group, p <0.05. Meanwhile, the average VEGF level in the MD-KLM group NTT was significantly higher than the MD-JW and MD-KLM groups, p < 0.05 (Fig. 2). And the mean VEGF level in the MD-JW group was significantly higher than in the MD-KLM group, p <0.05. From the results of this analysis it can be concluded that giving 2 ml of NTT honey had a better effect on stimulating angiogenesis than giving Java honey and Kalimantan honey.

This study used an experimental research type and a cross-sectional approach with four treatment groups, each group consisting of 6 mice that were treated and then assessed by calculating the comparison of the size of the wound area measured every day with the initial size of the wound, microscopic observation for examination VEGF immunology from the wound, the calculation of the number of leukocytes in blood samples and the calculation of the number of bacteria in the rat wound swab using the dilution method. Each treatment group was treated for wounds every day, macroscopic wound monitoring was carried out for seven days as well as observation of infection and examination of VEGF levels on the 7th day.

Honey is an alternative that can provide a faster effect on wound healing by stimulating tissue growth and the effect of epithelialization. The role of honey in wound healing is also supported by anti-inflammatory and antioxidant activities, and the ability to stimulate the removal of dead tissue [15] . Honey can inhibit the growth of bacteria around the wound, fungus is not susceptible to growing in honey. The spectrum in honey can inhibit the growth of gram-positive and gram-negative bacteria as well as aerobic and anaerobic bacteria [13].

Honey has a thick consistency and comes from flower nectar that has been processed by bees. The normal wound healing process is generally a chain process of coagulation, inflammation,

cell proliferation, and tissue remodeling [14]. *Vascular Endothelial Growth Factor* (VEGF) functions as a protein signal in the process of vasculogenesis and angiogenesis (new blood vessel formation). VEGF starts the regeneration process through the process of recruitment and differentiation of stem cells into endothelial cells that make up blood vessels [16].

In the initial phase of the inflammatory reaction, neutrophils and macrophages will enter the injured or injured tissue due to various chemotactic factors [11]. These cells will produce *Reactive Oxygen Species* (ROS) which can provide both beneficial and detrimental effects on the surrounding tissue. Apart from being produced by neutrophils, ROS which can provide a bactericidal effect are also produced by cells that are undergoing proliferation and have an important role in intracellular signaling in response to various extracellular stimuli, for example Hydrogen peroxide will be seen in limited quantities and induces *Vascular Endothelial Growth Factor* (VEGF) in the wound healing process which will be expressed in keratinocytes and also support the increase in angiogenesis. Conversely, excessive ROS production can cause tissue damage and interfere with the wound healing process [18]. The enzyme phosphotyrosine phosphatase and low molecular weight antioxidants such as glutathione play an important role in cellular redox regulation of cellular homeostasis which occurs because excessive ROS production can disrupt the function of communication between cells and ultimately affect the wound healing process [19]. The body itself has several antioxidant and redox systems to protect itself against damage caused by oxidative stress [12].

In this study the treatment group that provided optimal results for wound healing was in the K3 group (Flores Forest Honey, NTT), where the average healing rate was obtained (118.7 ± 29.01). This difference is suspected that the activity of honey in wound healing is also influenced by the characteristics of the honey itself and is caused by the species of honey-producing bees/wasps, geographical location, surrounding vegetation, and the process of making and storing it [9].

Based on Asroel's research, 2020 states that giving honey can increase VEGF in preecalmsia model rats [20]. The increase in VEGF expression in the administration of forest honey showed an increase in neovascularization compared to the control group. Forest honey is considered as one of the candidates among natural ingredients which according to data accelerates wound healing after palatoplasty surgery because of its high flavonoid content. Flavonoids are known to have anti-inflammatory and antioxidant properties and induce VEGF [17].

The results of all ANOVA test scores obtained $p=0.043$ ($p<0.05$), which meant that there were significant differences in the results in the variable group, and continued with the *Post Hoc* LSD follow-up test and obtained $p<0.05$, which means that there were significant differences in each each variable group. From the results of the research conducted, all treatment groups had an influence on the stimulation of angiogenesis in mice wounds. Honey originating from NTT has a better effect than honey originating from Java and Kalimantan.

IV. CONCLUSIONS

Based on the results of the study concluded, that administering honey from NTT can increase *Vascular Endothelial Growth Factor Levels* (VEGF) was better than honey from Java and Kalimantan in healing acute wounds infected with bacteria in BABL/C mice.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Eko Naning Sofyanita, Arya Iswara and Ahmad Riadi.
Methodology: Eko Naning Sofyanita and Arya Iswara.
Investigation: Eko Naning Sofyanita and Arya Iswara.
Discussion of results: Eko Naning Sofyanita, Arya Iswara and Ahmad Riadi.
Writing – Original Draft: Eko Naning Sofyanita
Writing – Review and Editing: Eko Naning Sofyanita and Arya Iswara.
Resources: Arya Iswara.
Supervision: Arya Iswara and Ahmad Riadi.
Approval of the final text: Eko Naning Sofyanita, Arya Iswara and Ahmad Riadi.

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