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THE EFFECTIVENESS OF INDONESIAN HONEY STIMULATE FIBROBLAST CELL VIABILITY AND MIGRATION THAT COULD POTENTIAL PROMOTE WOUND HEALING

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Abstract

Wound healing is a complex event involving both cellular and molecular activities. Fibroblasts play an important role and are keys to wound healing through cell proliferation and migration. Honey has anti-microbial, anti-oxidant, anti-inflammatory properties, which are used for various benefits such as wound healing. This study aims to explore the effect of honey on the viability and migration ability of fibroblast cells. The method used is the viability test using the MTT Assay calculated by the formula for the percentage of cell viability. Migration test using In Vitro Wound Scratch Assay. The results of the migration test images were analyzed using ImageJ. Giving honey doses of 0.5% and 0.1% increased cell viability and migration after 24 hours of intervention. Decreased cell viability after 48 hours of treatment, but there was a difference in the meaning of honey 1%, 0.5%, and 0.1% compared to control. Honey doses of 1%, 0.5%, and 0.1% increased fibroblast cell migration compared to control. The lowest honey increases the viability and migration of fibroblasts so that the possibility of wound healing.

Keywords: *Honey; Fibroblast Migration; Wound Scratch Assay; Wound Healing*

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1. Introduction

The skin is a protection for the human body from infection, wounds, and cuts. Wounds can be described as a breakdown of the anatomical and functional integrity of the skin's epithelial tissue. Types of acute, chronic, dry, or infected wounds can develop due to various physical and mechanical factors or different reasons such as animal bites, sharps injuries, and trauma (Dreifke et al., 2015; Mekonnen et al., 2013). Wound healing has various processes such as coagulation, inflammation, collagen production, and epithelial formation. Once the platelets contact the collagen in the wound area, they release clotting factors and growth factors. After that, neutrophils migrate to the wound site and remove microbes from the damaged tissue. Macrophages also contribute to this phase. Microorganisms can cause the release of pro-inflammatory cytokines such as interleukin-1 and TNF-alpha in the inflammatory phase. If this state persists, the wound may enter a chronic condition and fail to heal angiogenesis (Gonzalez et al., 2016; Lodhi et al., 2016)

Chronic wounds are a significant economic burden and cause enormous productivity losses for society and the medical system. Therefore, research on wound care products is still being developed to find alternative treatments expected to accelerate chronic wound healing and have economic value. Alternative materials can use plant, animal, mineral materials that can be used singly or combined for prevention and treatment (Fink, 2002).

Some naturally available ingredients such as honey and aloe vera are reported to be used to manage wound healing. Honey has been used for centuries to treat fever, pain, and treat wounds. Honey, in addition to having the effect of reducing pain, also can reduce inflammation and ROS production, to modulate the production of tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin (IL-6) especially maintaining the value of low pH (around 3.5) (Weiß et al., 2017). In addition, honey has antibacterial activity and stimulates fibroblast proliferation and angiogenesis, promoting an experimental remodelling phase (Martinotti & Ranzato, 2018; Saikaly & Khachemoune, 2017). Angiogenesis is an essential factor in wound healing remodelling. Anti-microbial and anti-inflammatory substances can prevent excessive TNF-α and IL-6 to accelerate the inflammatory phase of wound healing.

This study was conducted to explore the potential of honey on wound healing in vitro models.

The urgency of the study is to assess the effect of honey on wound healing so that it becomes one of the choices or alternative natural ingredients that can be used as wound care.

2. Method

Collection and Preparation of honey

The honey sample was obtained from the PKU Muhammadiyah Kitamura Clinic in Pontianak from the Putusibau forest, West Kalimantan. Honey was dissolved in Dulbecco's Minimal Essential Medium (DMEM, Gibco). Our previous research carried out the Preparation of honey solutions of various concentrations (Rizqi et al., 2019).

Cell Culture

This study used the NIH 3T3 cell line obtained from the Pharmacology and Therapeutics Laboratory, Faculty of Medicine, Gadjah Mada University. Fibroblasts were cultured normally in Dulbecco's Modified Eagle Medium (DMEM). DMEM medium was added 0.5% fungizone (GIBCO®, Grand Island, NY, USA), 1% penstrep 10% fetal bovine serum (FBS, Sigma), and incubated at 37oC in 5% CO2. Cell culture results were observed and replaced with DMEM media 3 times a week.

Fibroblast 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide [MTT] Assays

NIH3T3 fibroblast cells were put into 96 well microplates, each 100 L/well as much as 1 x 104. Cells were incubated for 24 hours under starvation. Treated with each combination concentration, then set again for 24 hours and 48 hours. The wells were added with 100 L of new media and 10 L of MTT reagent (10 L/100 L per well), then incubated for 4-6 hours in a CO2 incubator at 37°C. Each well was added with 100 L sodium dodecyl sulfate (SDS) 10% in 0.01% HCl. Incubated at room temperature for 12 hours or overnight. The microculture well was then read for absorbance using an ELISA reader at a wavelength of 595 nm. Calculation of proliferation by calculating cell viability is the percentage of cell viability = (Absorbance Treatment–Absorbance Media) / (Absorbance control cells–Absorbance Media) x 100%.

In Vitro Wound Scratch Assay

NIH3T3 cells were grown to total cells in 35 mm plates with a complete medium. Cell suspensions were produced in 24 wells microplate with a fibroblast cell density of 5x104 cells/mL in each well and incubated for 24 hours in a complete medium (DMEM with 25 mM glucose, 10% FBS, 1% Penstrep, 0.5% Fungizone). Make scratches on the bottom surface of the well using the yellow tip. Cells were then stored in an incubator treated in culture media. Wound streaks are monitored under a microscope with appropriate images recorded using a digital camera. The distance between one side of the stroke and the other is calculated at specified intervals (μm) using ImageJ software for each image. By comparing the images from 0 hours to the last time point, the distance of each stroke is obtained.

Statistical Analysis

The collected data were tested for normality using the Shapiro-Wilk test. If normally distributed, it was followed by one-way ANOVA with a confidence level of 95%. a post hoc multiple comparison LSD test was performed To compare the differences in each group. The results are said to have a significant difference if the p-value <0.05. If the data is not normally distributed, use the Kruskal-Wallis non-parametric test followed by the Mann-Whitney test.

3. Results and Discussion

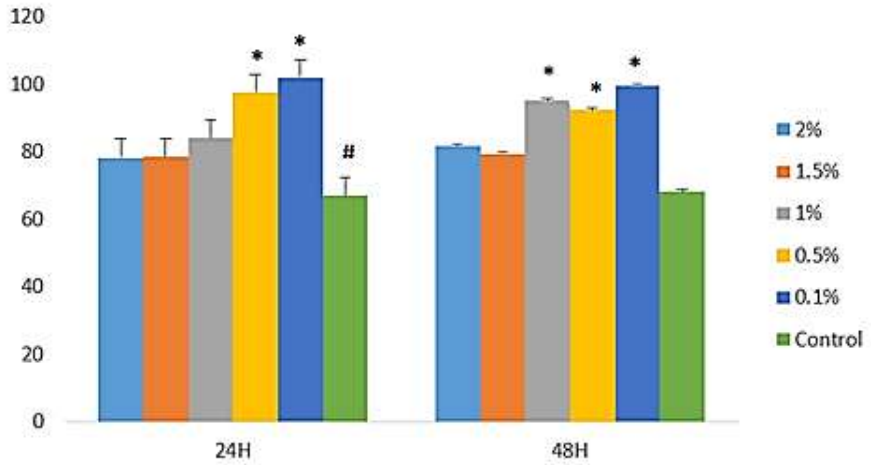

Figure 1. Cell viability results after being given honey at 24 hours and 48 hours. ANOVA test in 24 hours (p= 0.000) and 48 hours (p= 0.007). 24-hour Post Hoc LSD Test; * <0.05 compared to all honey and control groups; # <

0.05 compared to the honey group. 48-hour Post Hoc LSD Test; * <0.05 compared to 2%, 1.5% honey group and control

Assessment of fibroblast viability after 24 hours and 48 hours of intervention can be seen in Figure 1. The increase in viability was seen with the administration of the smallest dose of honey after 24 hours. The amounts of 0.5% and 1.5% honey showed a significant difference compared to the control ($p<0.05$). There was an increase in cell viability after 48 hours of honey treatment. The doses of 1%, 0.5%, and 0.1% were significantly different from the control. These results indicate that honey with the smallest amount can increase the viability of fibroblast cells

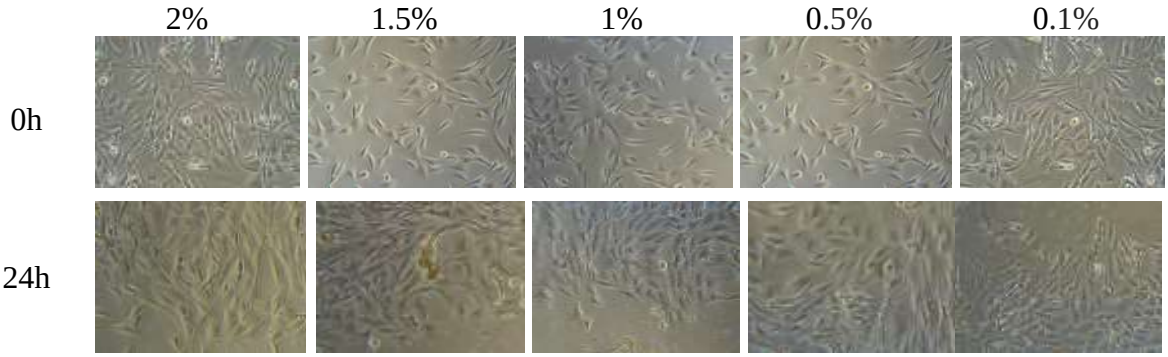

Figure 2. The morphology of fibroblasts after being given honey at 24 hours. Honey did not change the cell morphology. Images were taken at 40x magnification with a Miticam microscope

Observation of fibroblast morphology is needed to determine the condition of healthy cells. The results of the observations can be seen in Figure 2. The shape of normal fibroblasts can be elongated, ovoid or multipolar. After 24 hours of honey treatment, it was observed that there was no change in fibroblast morphology. These results illustrate that the administration of honey does not cause toxicity to cells.

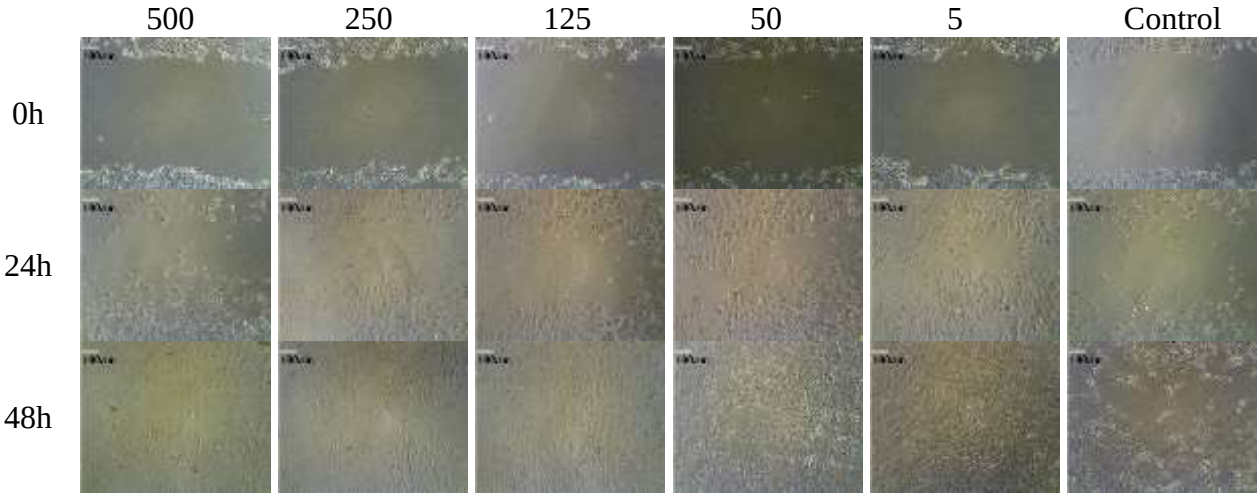

Figure 3. Results of fibroblast cell migration starting from the beginning of the In Vitro Wound Scratch Assay, 24 hours, and 48 hours after honey intervention

Cell migration testing after being given honey for 24 hours and 48 hours can be seen in Figure 3. At 0 hours, there were no cells in the scratch area. After 24 hours of treatment, the cell movement began to appear towards the centre of the scratch area. After 48 hours of treatment, 0.5% and 0.1% honey concentrations seemed to have covered all scratches.

These results also showed a significant difference between the 0.5% and 0.1% honey groups compared to the control group after 24 hours of treatment (figure 4). Cell migration increased significantly in the 1%, 0.5% and 0.1% honey groups compared to the control group after 48 hours of treatment

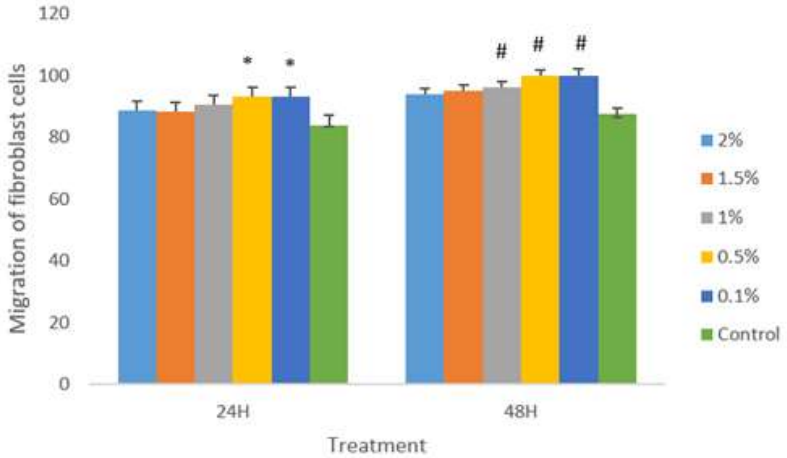

Figure 4. Migration of fibroblasts 24 hours and 48 hours after being given honey. ANOVA test in 24 hours (p= 0.023) and 48 hours (p= 0.000). LSD Post Hoc; 24 hours *(p<0.05) compared to the honey group1 2%, 1.5%, 1% and control cells; 48 hours # (p<0.05) compared to 2%, 1.5% honey group and control cells

The physiological process of wound healing is divided into four phases; hemostasis, inflammation, proliferation, and remodelling (Wang et al., 2018). Fibroblasts are essential cells in the wound healing process. After the inflammatory phase, fibroblasts are responsible for breaking down the fibrin matrix replacing the ECM structure and then promoting cell proliferation and migration to the wound area (Tracy et al., 2016). Therefore, knowing the increase in cell viability, proliferation and migration are very important in wound healing.

Measurement of viability with the MTT test is needed to determine cell metabolism activity after being given treatment (Norazzila et al., 2017). The effect of honey on cell viability depends on the dose given (figure 1). The results of increased and decreased cell viability at specific doses showed a multipolar pattern of honey. The maximum increase in viability was found in 0.1% honey after 24 hours and 48 hours of treatment. Giving honey with different effects was also reported by previous studies (Kassim et al., 2010). This increase in viability is attributed to the phenolic compounds present in honey. Phenolics will be the main active component of honey, producing effects depending on its concentration (Kassim et al., 2010).

The administration of honey did not affect the cell morphology observed microscopically. Normal fibroblasts appear elongated and multipolar in shape. Giving honey is not toxic to cells and increases cell viability. These results are similar to our previous study that honey is not toxic to fibroblast cells (Rizqi et al., 2019). These results are also supported by earlier studies that honey does not change cell morphology and can improve wound healing (Nordin et al., 2018).

Fibroblast migration is an essential process in wound repair. Fibroblasts develop and migrate to the injured area, synthesize new extracellular matrix, and play a role in wound healing (Kanazawa et al., 2010). Our results showed that honey administration increased fibroblast cell migration. This can be seen from the speed with which fibroblasts move to cover the wound area 24 hours after treatment (figure 3). Other studies have also reported that the administration of honey can facilitate fibroblasts to unite a matrix that is useful for wound healing (Chaudhary et al., 2020). Honey has actual content, such as hyaluronic acid, which plays a role in fibrosis repair (Zeweil et al., 2020). In human skin, extensive hyaluronic acid fragments result from enzymatic and reactive oxygen functions as a stimulus for cell migration and wound healing angiogenesis (Tolg et al., 2014). The content of honey that functions as an antioxidant and anti-inflammatory, one of which is flavonoids. Flavonoids trigger macrophage cells to produce TGF-β, which will increase cell number, proliferation and differentiation of fibroblast cells (Yuslianti et al., 2016). Honey is also helpful as an antimicrobial, works by reducing microbial infection and facilitating wound healing (Abd Jalil et al., 2017)

4. Conclusions and suggestions

Honey has a beneficial effect by increasing the viability and migration of fibroblast cells. Giving honey did not change the morphology of fibroblasts. The conclusion is that the smallest dose of honey has the potential to improve wound healing by increasing the proliferation and migration of fibroblasts.

5. Acknowledgments (if any)

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