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The Effectiveness of 80% Kefir Gel Against The Overview The Number of Fibroblasts in Healing Cuts Mice (*Mus Musculus*)

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Abstract
Several sources cause skin tissue damage. Kefir probiotics, made from fermented cow's milk, are believed to remove scars during use. Kefir involves a series of immune processes, acute and chronic inflammatory processes, cell division cell migration, chemotaxis, and various differentiations. This research determines the histopathological fibroblast cells in the wound healings in mice (Mus musculus) given 80% kefir probiotic. The method of research was the posttest only control group experimental design. Mus musculus aged eight weeks with a bodyweight of 18-40 grams were taken in two groups randomly with 32 Mice. The result is that fibroblasts in the negative control group had a smaller mean of 10,25 compared to the mean number of fibroblast cells in the 80% Kefir group, which was 25,18. Thus, it was proven that the effectiveness of Kefir probiotics of wound healing in mice (Mus musculus) was given 80% kefir probiotic (p=0,000 <0.05). There is an effect of kefir probiotic on the healing of cuts in mice based on the calculation of the number of fibroblast cells.

Keywords : Kefir Gel; Fibroblasts; Healing

INTRODUCTION

The skin plays a role in the human body, which acts as a body protector against mechanical, thermal, physical damage, harmful agents, prevents moisture loss (dehydration), protects against the harmful effects of UV radiation from the sun, acts as a sensory organ, regulates temperature control and plays an essential role in the body's functioning. the immunological system, and produce vitamin D(Dewi et al., 2018; Huseini et al., 2012; Johnson et al., 2018).

Injury to the skin is a form of tissue damage caused by several sources such as heat (chemicals, hot water, fire, radiation, and electricity), the result of medical action, and changes in physiological conditions)(Knackstedt et al., 2020; M. R. Prado et al., 2015). In general, there are two types of wounds, open wounds and closed wounds. An open wound is a wound on the surface of the skin resulting in external bleeding. According to the Centers for Disease Control and Prevention 2016, in 2011, the prevalence survey results found about 157,500 incidents of infection due to surgery with patients hospitalization. Therefore wounds cannot be considered as easy problems. The purpose of treating wounds is to prevent trauma (injury) to the skin, mucous membranes or other tissues that can damage the skin surface. According to the World Health Organization (WHO), 80% of Asian and African countries use traditional methods, namely herbal medicines, to treat wounds because they are cheaper, easy to obtain and have common side effects(Huseini et al., 2012; Nam et al., 2021).

Wound healing is a complex process that occurs physiologically in the body. Wound healing consists of several phases, namely inflammation, proliferation and maturation. Wound healing is necessary to regain intact body tissues. Several factors play a role in accelerating the healing process; internal factors and external factors. External factors that can accelerate wound healing are using topical drugs and synthetic and natural medicines using synthetic and herbal medicines(M. R. Prado et al., 2015; Salimi & Mohammadipanah, 2021; Zaetun et al., 2018).

Kefir probiotics, made from fermented cow's milk, kefir products are now widely used as a wound healer; apart from being a wound healer, it can also remove scars. The mechanism of action of kefir in wound healing involves a series of immune processes, both acute and chronic inflammatory processes, cell division, cell migration, chemotaxis and differentiation of various cell types, which ultimately result in structural and functional tissue recovery(Dimidi et al., 2019; Huseini et al., 2012). Kefir contains normal flora that can stimulate the innate immune response for defence against pathogenic bacteria that aggravate skin wounds. The lactic acid in kefir fermentation can also inhibit the proliferation of various species of pathogenic bacteria. *Kefir polysaccharide* is an anti-inflammatory that plays a significant role in the wound healing process(Lolou & Panayiotidis, 2019; Meire Dos Santos Falcão et al., 2018).

The wound healing process consists of 4 phases: hemostasis, inflammation, proliferation, and remodelling. The crucial phase of bacterial infection occurs during the inflammatory phase. If an infection occurs, the process of proliferation and remodelling will be hampered and slow wound healing. Therefore, the action of kefir on wound healing which begins in the inflammatory phase, is decisive for inhibiting the infection reaction and accelerating the wound healing phase(Wilkinson & Hardman, 2020; Yildiz et al., 2019).

Kefir contains a polysaccharide called kefiran. Kefiran is a heteropolysaccharide with the main component of glucose and galactose. Kefiran is mainly produced by *Lactobacillus kefiranofaciens*. Kefiran is an immunomodulator, re-epithelialization activator, and antioxidant that accelerates wound healing. In addition to the content of polysaccharides, hyaluronic acid, lactic acid, acetic acid work in the proliferative and maturation phases of wound healing, resulting in accelerated scar tissue formation and regeneration of wound tissue. Another function of hyaluronic acid, lactic and acetic acid, is anti-inflammatory and antimicrobial, supporting the healing reaction acceleration (Lolou & Panayiotidis, 2019; Oryan et al., n.d.; M. R. Prado et al., 2015; Safitri, 2016).

Research on kefir as an ingredient for accelerating wound healing has been done but is still limited. In the last three years, even the latest research about 80% kefir gel on wound healing has not been found. Previous research by Dewi (2018) stated that 70% kefir gel accelerates wound healing reactions.(Dewi et al., 2018). Based on this explanation, the author feels that 80% kefir gel research is essential to accelerate the wound healing process in cuts.

METHOD

This type of research was experimental with the "the posttest only control group design". The sample in this study was mice (mus musculus) aged 8-12 weeks, 250-300 grams There were two groups, negative control group (K-) and treatment group 1 (P1) for 14 days. The materials used in this study were 80% kefir gel produced by PT Putkayo International, medicine for anesthesia white mice (ketamine), alcohol swab, clean water for drinking white mice, pellets for eating white mice, and rice husks for drum mats. Before the research, the hair around the back of the slashed mice was shaved according to the desired area. Then, use anesthetic procedure using lidocaine 0.2-0.4ml/KGB for superficial surgery. The incision wound in the negative control group (K-) was given treatment. The incision was only cleaned with sterile gauze, and distilled water was not provided any therapy. In treatment group 1 (P1), the incision was treated with 80% kefir probiotic until it closed—the entire wound surface. After applying, each is covered with sterile gauze. The treatment on the cut wound was carried out once a day for 14 days according to the length of the normal wound healing process reaching the proliferative phase. The calculation of the number of fibroblasts was seen with a binocular microscope (Olympus Type CX31) magnification of 400x, and the analysis was carried out with three fields of view and then counted the number of fibroblasts in each area of view so that it was visible. Data analysis used descriptive analysis, an overview of the characteristics of fibroblasts. The normality test was tested with the Shapiro-Wilk test for fibroblasts and collagen fibers. The distribution of data is normal with $p>0.05$. The homogeneity of the data was tested with Levene's test. The data tested were fibroblasts and collagen fibers. The data is homogeneous with $p>0.05$. If the data distribution is not normal, then the data is analyzed by Mann Whitney.

The code of ethics for this research is: 026 /ETIK-FKUNBRAH/03/02/2021

RESULTS AND DISCUSSION

Overview of the average number of fibroblasts in the healing of incisions in mice (Mus musculus) given 80% kefir probiotics. Based on the data obtained, the histopathological description in the form of the number of fibroblast cells in Wound Healing of Mice (Mus musculus) given 80% kefir probiotics in this study with a description of the results as follows.

Table 1. The Data of Fibroblast

Groups	Fibroblast
	Mean±Deviation standard
P1 (Kefir 80%)	25,18±8,19
K- (control)	10,25±6,26

The data at table 1 shows the average number of fibroblast cells in mice. The 80% Kefir probiotic treatment group has a higher average compared to the average fibroblast cells in the control group.

Kefir has many benefits for skin health. Kefir contains active standard flora cultures of various strains of microorganisms, which help fight against pathogenic microorganisms. In addition, kefir contains several active compounds such as polysaccharides, peptides, and organic acids that are effective in inhibiting the work of enzymes in the process of forming skin pigment (melanin) and effective in overcoming the damage caused by free radical compounds(Huseini et al., 2012; Sulmiyati et al., 2019).

Kefir Effective for Wound Healing This is because Kefir contains vitamin C, which plays a role in cell differentiation, collagen synthesis and increasing fibroblast proliferation. The vitamin C content is significant in the proliferative phase causing interaction with macrophages to produce growth factors. These growth factors contribute to wound healing by stimulating fibroblasts to produce more collagen, filling the wound area and boosting the immune system. This good immune state can improve the function of the immune system, thereby increasing the proliferation of fibroblasts(de Lima et al., 2018).

In this study, the administration of 80% Kefir had a significant difference in increasing the density of fibroblasts in mice compared to the negative control group. The higher number of fibroblasts in the group with 80% Kefir than the group without treatment. This increase in fibroblast density is thought to be because Kefir also contains CO2, diacetyl, acetaldehyde, and hydrogen peroxide as well as bacteriocins, which are protein compounds that show antibacterial activity against bacteria, the chemical composition of Kefir depends on the milk used as raw material, namely protein 3.91% lactose 2, 88%, 2.45% fat and 0.94% ethanol and Kefir have a pH of 3.77-4.19 with an acidity degree of 1%(de Lima et al., 2018; Dimidi et al., 2019; Huseini et al., 2012).

Fibroblasts work from the 5th to the seventh day of wound healing. Fibroblasts work from the inflammatory phase to the remodelling phase. Fibroblasts deposition extracellular matrix component (ECM), wound contraction and remodelling the new ECM. Fibroblasts work to form new collagen and glycosaminoglycans in the wound. These newly formed proteoglycans serve as centres for wound healing. Simultaneously with proteoglycan action, reepithelialization occurs from the periphery and adjacent edges of the wound. The epithelialization formed first is a thin epithelial layer on the outside and will get thicker along with the wound healing process. The next stage is the formation of angiogenesis and neovascularization(Addis et al., 2020; Meire Dos Santos Falcão et al., 2018; Wallace et al., 2021).

The data from calculating the number of fibroblasts in the rats after treatment in each group were then analyzed by normality test of the data using Shapiro-Wilk with a description of the results as follows:

Table 2. Normality Test

Group	Sig.	Normality Test
P1	0,001	-
K-	0,270	+

Based on the data in table 2, it can be concluded that the bivariate analysis used was non-parametric Mann Whitney. Furthermore, a non-parametric Mann Whitney test was carried out to determine the effectiveness of kefir probiotics on the description of the number of fibroblasts in wound healing in mice (*Mus musculus*) with a confidence level of 0.05 with a description of the results as follows:

Table 4. *Mann-Whitney* test

Groups	N	*Asymp. Sig. (2-tailed)*
K-	16	0,000
P1	16	

Based on Table 4, the study results showed the effect of giving kefir probiotics on wound healing in mice based on the number of fibroblast cells.

The effect of kefir 80% could be seen at figure 1, describe the increase in the number of fibroblasts in mice with 80% kefir gel.

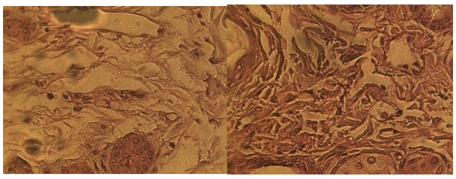

Figure 1. Histology of Fibroblast Cell

The previous research by Safitri, regarding the Effect of Giving Kefir Ointment on the Number of Polymorphonuclear Cells (PMN) and Fibroblasts in Healing Laparotomy Incision Wounds in Rats (Rattus norvegicus) obtained topical kefir administration significantly ($p<0.05$) affect the number of fibroblasts. Therefore, the effective dose for the therapy of laparoscopic incisions is kefir ointment with a concentration of 40%, which can restore the number of PMN cells and two fibroblasts in normal conditions. In addition, the content of compounds in kefir ointment, namely polysaccharides, Cu, and Zn, can reduce the migration activity of polymorphonuclear neutrophil cells, and vitamins C, E, and Fe can increase fibroblast proliferation in collagen synthesis(Lolou & Panayiotidis, 2019; Safitri, 2016).

At the beginning of wound healing, fibroblasts have contractile abilities and are called myofibroblasts, which will cause the wound edges to be pulled and then closed so that the two wound edges will stick together. As healing progresses, the number of fibroblasts increases. Fibroblasts play a role in the synthesis, deposition, and remodelling of the extracellular matrix. After migrating to the wound site, fibroblasts begin to synthesize the extracellular matrix. Proliferating fibroblasts accompany these vessels and begin to accumulate collagen. In the proliferative phase, for 3 to 5 days, a particular type of tissue that characterizes healing is called granulation tissue(Amita & Balqis, 2017; Farag et al., 2020; M. R. M. Prado et al., 2016)

Kefir is proven to be effective as a wound healing in mice, and this is also because kefir ointment contains vitamin C, which plays a role in cell differentiation, collagen synthesis and increasing fibroblast proliferation. The vitamin C content is significant in the proliferative phase causing interaction with macrophages to produce growth factors. These growth factors contribute to wound healing by stimulating fibroblasts (connective tissue cells) to produce more collagen, filling the wound area and boosting the immune system. This good immune state can improve the function of the immune system, thereby increasing the proliferation of fibroblasts. In addition, kefir gel also contains proteins that increase the proliferation of fibroblasts, thereby increasing the synthesis, accumulation, and remodelling of collagen(de Lima et al., 2018; Knackstedt et al., 2020).

Fibroblasts in wound healing are very important because the more fibroblasts formed during the healing process, the faster wound contraction and wound healing will occur. However, fibroblasts at the wound site will begin to decrease and return to normal around day 14 when the extracellular matrix in the wound has reached a tensile strength that is almost the same as the surrounding healthy tissue(Baniasadi et al., 2021; M. R. Prado et al., 2015).

This study found that giving kefir gel 80% increased the number of fibroblasts on day 7, which is the peak of fibroblast work in the wound healing process. However, the study did not look at the effect of accelerating fibroblast activation by giving 80% kefir gel, so it is uncertain whether 80% kefir gel works to accelerate the wound healing process by increasing the number of fibroblasts or accelerating work activation and increasing the number of fibroblasts.

CONCLUSION

The increase in the number of fibroblasts in mice with 80% kefir gel proved that 80% kefir gel was effective against wound healing in mice (mus musculus)

Further research should examine the effect of 80% kefir gel on fibroblasts on days 3, 5, and 7 to determine whether kefir gel accelerates fibroblast activation in the wound healing process

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