IN VIVO STUDY OF KEFIR GEL PROBIOTICS ON WOUND HEALING

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IN VIVO STUDY OF KEFIR GEL PROBIOTICS ON WOUND HEALING

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Abstract
Kefir accelerates the wound healing reaction of cuts starting from the hemostasis phase to proliferation. Kefir is a readily available probiotic. However, research on kefir as a therapy to accelerate wound healing is still limited. The purpose of this study was to examine the effect of 80% kefir gel in vivo on the acceleration of wound healing in mice (Mus musculus).This research is a post-test-only control group design. The number of mice used was 32 mice which were taken randomly and divided into two groups (experimental and control group). Kefir 80% was given for 14 days, then the wound healing was measured. The results of the study conclude that wound healing in the 80% kefir gel group started on day 9, three days faster than the control group. Wound healing includes wound size and wound healing time.Conclusion: Kefir gel with a concentration of 80% can accelerate the wound healing process
Keywords: *Kefir, Wound Healing, Probiotics.*

INTRODUCTION

The wound healing process requires both cellular and biochemical actions. Matrix metalloproteinase (MMP) and tissue inhibitor metalloproteinase (TIMP) play an essential role in wound healing. MMP is an enzyme that breaks down proteins in the wound bed. Among the 23 types of MMP, four types play an important role in wound healing, namely MMP-1, MMP-2, MMP-8, MMP-9. Inflammatory and wound cells produce a latent form of MMP, called pro-MMP. This latent form is activated via membrane-type MMP (MT-MMP) or other proteinase enzymes. Active MMP has a role in every phase of wound healing, namely the inflammatory phase, the proliferative phase, and the maturation phase.[1]

The typical phase of wound healing can be predicted according to the time of its development. Cells that play a role in all these processes are macrophages, secrete pro-inflammatory and anti-inflammatory cytokines and growth factors. In fibroblasts, they will synthesize collagen, which affects the tensile strength of the wound and fills the wound tissue back to its original shape, followed by other cells. Skin keratinocyte cells divide and migrate to form re-epithelialization and cover the wound.[2]

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Probiotics as a wound-healing therapy, as evidenced by previous studies, probiotics used are honey as wound healing therapy; Honey has good content for health because honey contains antibacterial, anti-inflammatory, and antioxidant properties, making it very suitable for wound healing.[3] Probiotics help wound healing through epithelial cells' activation mechanism, stimulating fibroblasts, and increasing collagen synthesis. Lactobacillus reutari accelerates the wound healing process by increasing the regulation of the neuropeptide hormone oxytocin. Wound healing is related to the induction of Tumor Growth Factor-β (TGF-β), Endothelial Growth Factor (EGFR), and Insulin Growth Factor (IGF) expression.[4], [5] Kefir is a probiotic made from cow's milk added to a kefir starter in kefir grains or kefir grains. Traditionally, making kefir is done by heating milk (pasteurized) at a temperature of 85-90^{0}C, then cooled to room temperature of 18-22^{0}C and put 3% kefir grains stirred with an average incubation of 18-24^{0}C.[6]

A study found probiotic organisms, polysaccharides, peptides, organic acids, especially lactic acid, and even the lactic acid properties of fermented kefir could be efficacious for maintaining skin health or treating damage to the skin, such as hyperpigmentation, premature aging, acne-prone skin, and efficacious in accelerating wound healing and wound infection on the skin.[6] A previous study also supports this that kefir ointment with a concentration of 40% can be used as a therapy for laparotomy incisions.[7] Currently, medicines have shifted to using natural remedies to accelerate wound healing because they are proven safe, do not cause side effects, and are more economical. People still consume many probiotic foods as a medicine for wound healing, one of which is "Kefir."

Research on the effect of kefir gel has been investigated. The result is that kefir gel accelerates the wound healing process. However, in the previous study, the concentration of kefir studied was 40%; this concentration was felt less because, at the end of the study, scars were still visible with almost the same size as the control group although without swelling and redness around the scars. Research with the largest concentration has never been studied before whether it gives a better effect or not. This research is crucial to fill the information gap in previous studies, to see the effect of 80% kefir gel on wound healing. This study aims to examine the effect of 80% kefir gel on the acceleration of the wound healing process of cuts.

METHODE

This research was experimental post-test only control group design method and using animals with a randomized. This research was carried out in the pharmacology laboratory of the Faculty of Pharmacy, Andalas University. The surgical instrument used in this study were: surgical scissors, anatomical tweezers, medical gloves, stainless-button wax surgical board, labels, markers, caliper, cotton buds; this preparation material: Aquadest, alcohol 70% and kefir gel 80%.

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In Vivo Procedure

1. *Animal Preparation*
   The population in this study were white mice aged eight weeks with a bodyweight ranging from 18-40 grams. Mice were acclimatized for seven days, with a room temperature of 26-27^{0}C and 76-87% humidity.
2. *Experimental procedure*
   Thirty-two rats were randomly divided into two groups, the treatment group (P1) and the control group (C). Make a wound by an incision 1 cm long with a depth of 0.2 cm or up to the dermis layer on the back using a sterile scalpel. Perform cleaning by flowing aqua dest until the bleeding stops. In the P1 group, the wound was treated using 80% kefir gel administered once a day at the same hour for 14 days. In the wound control group, the natural wound healing process was observed. The length of wound healing is calculated starting on the 7th and will be measured until the 14th-day post-injury. When the wound is completely closed, the calculation of the length of the wound will be stopped.
3. *Statistical Analysis*
   Bivariate data analysis was carried out on the length, width of the wound, and the difference in the speed of wound healing pre-treatment and post-treatment. The data is significant if the significance level of <0.05.

This research has been registered with the research ethics committee of Baiturrahmah University Padang with ethical approval number 027/ETIK-FKUNBRAH/03/02/2021.

RESULT AND DISCUSSION

In this study, it was found that there was an increase in wound acceleration of 42%, where the mean of wound healing given 80% kefir was reduced by three days compared to the negative control group. In the 80% kefir treatment group, there was a 43% reduction in scar length compared to the control group. A 41% reduction in scar width was found in the 80% kefir treatment group compared to the treatment group. The results of the bivariate test showed that the 80% kefir in vivo test on cuts reduced wound size and wound healing time compared to the control group (p<0.05). The reduction in size and duration of wound healing was on average 40% compared to the control group. The treatment results can be seen in Table 1 and Figure 1.

Table 1. Length, Width, and Time Speed of Wound Healing

Group	Length Wound (mm)	Width Wound (mm)	Speed Time (days)
Control (-)	1.95±0.19	0.64±0.11	12.44±0.63
P1	1.49±0.18	0.45±0.08	9.38±0.50
p-value	0.000 [a]	0.000 [b]	0.000 [a]

Exp: [a] Independent sample t-test; [b] Mann-Whitney test

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Figure 1. Wound Healing in 7 days and 14 Days of Treatment

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The study found a more significant effect on wound healing using 80% kefir. Acceleration of wound healing can be seen from an acceleration of healing of size of the wound and the time of wound. A significant effect of 80% kefir was seen on day nine by closing the wound, although the scar was still visible.

The wound healing process consists of hemostasis, inflammation, proliferation, and remodeling. Immediately after injury, the damaged blood vessels contract, and blood clots immediately prevent bleeding. The main contributor to hemostasis is platelets. Platelets play a role in hemostasis and coagulation. Platelets also play a role in activation by capturing immune cells directly in the eschar or releasing a chemokine attractant secretome during degranulation. In the next stage, an inflammatory phase activates resident immune cells, such as mast cells, Langerhans cells, T cells, and macrophages, by binding to pattern-recognition receptors to elicit downstream inflammatory pathways. The last stage is remodeling the Extra Cellular Matrix (ECM). Remodeling begins with the deposition of a fibrin clot. *Fibroblasts* are the primary cells that replace fibrin with hyaluronan, fibronectin, and proteoglycans and form mature collagen fibrils, which are then repaired.[8], [9]

The compounds in kefir ointment, namely polysaccharides, Cu, and Zn, were able to reduce the migration activity of polymorphonuclear neutrophil cells, and vitamins C, E, and Fe were able to increase the proliferation of fibroblasts in collagen synthesis, which was given to rats for healing of laparoscopic incisions.[9] The role of fibroblasts is also huge in the wound repair process, which is responsible for producing protein structure products used during the tissue reconstruction process. Fibroblasts (connecting tissue cells) migrate to the wound area starting in the first 24 hours after surgery. In normal soft tissue (without injury), exposure to fibroblasts is infrequent and usually hides in the supporting tissue matrix. After an injury occurs, fibroblasts will actively move from the tissue surrounding the wound into the wound area, then will develop (proliferate) and secrete several substances (collagen, elastin, hyaluronic acid, fibronectin, and proteoglycans) that play a role in reconstruction.[2], [10]–[12]

The effect of topical kefir is seen in the inflammatory phase of wound healing. During the inflammatory phase of wound healing, there is an increase in reactive oxygen species (ROS) in the wound area. The increase in ROS will be suppressed by kefir by binding ROS because kefir is an antioxidant.[13] The second effect of kefir during the inflammatory phase will increase the innate immune response by stimulating neutrophils, monocytes, lymphocytes, and platelets. Topical kefir will also increase re-epithelialization, increase collagen type 1 collagen deposition, and neo-vascularization, resulting in accelerated wound healing.[3], [14]–[16]

Kefir 80% concentration direct can accelerate the inflammatory phase in skin wounds. This is because the compounds in kefir ointment can reduce the migration activity of polymorphonuclear neutrophil cells so that the accumulation of excessive neutrophils can be reduced, tissue damage due to phagocytosis can also be reduced, the inflammatory process in the wound area takes place more quickly, and the resolution process occurs faster. Kefir also contains vitamin C, which plays a role in cell differentiation collagen synthesis and increases the proliferation of fibroblasts. The vitamin C content is significant in the proliferative phase causing interaction with macrophages to produce *growth*

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factors. *This growth factor* contributes 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[11], [17], [18]

Routine wound healing involves a series of immune processes, acute and chronic inflammatory processes, cell division, cell migration, chemotaxis, and differentiation of various cell types, resulting in structural and functional tissue recovery. Kefir contains normal flora that can stimulate the innate immune response for defense against pathogenic bacteria that aggravate skin wounds. In addition, the lactic acid in kefir fermenters can also inhibit the proliferation of various species of pathogenic bacteria, and kefir polysaccharides are anti-inflammatory, which plays a significant role in the wound healing process.[19]–[21]

Kefir gel was used for two weeks. The gel can reduce the percentage of wound size, reduce inflammation, increase the rate of epithelialization and scar formation. Kefir is better than the standard drug commonly used in treating burns, namely 1% silver sulfadiazine.[17] Vitamin C in kefir can help wound healing through oxidation with the cofactor Fe2+, which causes the release of several superoxide oxygen radical anions (O2-), which increase collagen synthesis. Kefir also has an immunomodulatory effect by influencing the activity and number of pro-inflammatory cytokines such as interleukins, TNF-a, and TLR-2.[22]–[24]

The previous research proves that changes in the microscopic picture of laparotomy incision wound healing in the skin tissue of Wistar rats an effective dose for the therapy of laparoscopic incisions is kefir ointment with a concentration of 40%, which can restore the cell count. PMNs and fibroblasts were in normal conditions[9]. Effect combination ointment of kefir and Aloe vera, a concentration of 37.5%, could reduce TNF- expression and improve collagen density with a reasonably good wound healing rate. the shows that the probiotic levels in kefir and aloe vera can accelerate wound healing in mice.[25], [26]

Previous study stated that topical kefir administration would reduce IL-1β and TGF-β1 expression at day 7. Kefir also reduced the expression of IL-1β at days 14 and 28 and stimulated bFGF at day 28. causes significant acceleration of wound healing on kefir.[13] Previous study stated that administration of fermented kefir for 96 hours in burn patients showed a significantly reduced inflammatory process and re-epithelialization at day 14 compared to 1% silver sulfadiazine, base gel, and untreated groups. The results of the burn germ culture in this study were Pseudomonas aeruginosa, so kefir inhibited the growth of Pseudomonas aeruginosa colonies which could inhibit the process of accelerating wound healing.[17]

Research with the content of other probiotics about the effectiveness of honey compared with povidone-iodine to healing cuts in mice obtained the results of the test the effectiveness of honey group 50% and povidone-iodine 10% compared with the honey group 100 % obtained $p<0.05$, where the healing time is faster in the 100% honey group than in the 50% honey group and 10% povidone-iodine, this is related to the physical effects of honey such as acidity, osmotic, chemical, and antimicrobial activity which are the primary sources in wound healing.[27]

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The content of probiotics plays an essential role in wound healing. This is also by other studies about the probiotic content, which showed that in Propionibacterium acnes, the test bacteria obtained a clear zone of 7.6 mm, the minor diameter compared to other test bacteria. This result is smaller than the significant inhibitory effect on staphylococcus lactic acid bacteria isolated from curd from Lintau.[28]

CONCLUSION

Kefir 80% in vivo accelerates the size reaction and recovery of wound healing. For further research, the researcher suggests researching the effect of kefir on accelerating wound healing, skin aging, and scar therapy

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