



## Comparison of Two Types of Gels in Improving Burn Wound

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### Abstract

**Objective:** Kefir are natural probiotic compounds with anti-inflammatory properties, which were tested in experimental burn injury. Kefir gels were prepared from an extract of continuously cultured kefir in Man, Rogosa and Sharpe Broth medium for 48 and 96 h. Their extracts were used for evaluation of antibacterial effect against *Pseudomonas aeruginosa* in standard sample (ATCC 27853) and samples taken from patients with burns. The antibacterial effect of different kefir extract was assessment of minimum inhibitory concentration and minimum bactericidal concentration. The density of bacteria and percentage of organic acids (lactic and acetic acids) were also determined.

**Materials and Methods:** Similar burn injuries were made on dorsal skin surface of 40 rats. The rats were divided into 5 groups of 8 rats each. The base gel, silver sulfadiazine ointment, kefir 48 h gel, kefir 96 h gel were applied twice daily. Burn wound area was measured at baseline, 1 and 2 weeks.

**Results:** Results indicated that by increasing the time of fermentation, concentration of lactic and acetic acid increased in orders of: Kefir 48 h < kefir 96 h, the end of the 2<sup>nd</sup> week the percentage of wound size were lowest in order of kefir 96 h gel < kefir grains 48 h gel < silver sulfadiazine 1% < untreated and based gel groups.

**Conclusion:** In conclusion, the kefir gel therapy was an effective therapeutic approach to improve outcomes after severe burn when compared with conventional silver sulfadiazine treatment.

**Keywords:** Burn, Kefir Gel, Wound Healing

### Introduction

Normal wound healing consists of a series of coordinated overlapping phases that involves acute and chronic inflammation, cell division, cell migration, chemotaxis and differentiation of numerous cell types (1,2). These phases are tightly regulated and results in wound healing and restoration of the structural and functional integrities of the damaged tissues (3,4). Although in modern burn wound management, topical antibiotics such as silver sulfadiazine dressing is mainly used (5,6), but due to its adverse effects, bacterial resistance and ineffective on healing process search for alternative compounds that speed the wound healing process is of an interest (7-9). However, the probiotic compounds may be of good choices. Probiotics are

single strain or a mixture of different organisms and are claimed to strengthen the immune system, reduced inflammation and speed wound healing process following accumulation of lymphocytes, macrophages and poly morphonuclear in place of injury (10,11). Kefir grains are a probiotic mixture of diverse spectrum of bacteria and yeasts (10). The microorganisms present in the kefir grains produce lactic acid (12). Such products due to antibacterial properties inhibit the proliferation of pathogenic microorganisms (13). The anti-inflammatory properties of polysaccharide present in the kefir extract also influences wound healing process (14,15). Kefir grains also stimulate innate immune responses in defense against pathogens (11,16). However in this study, the effects of different

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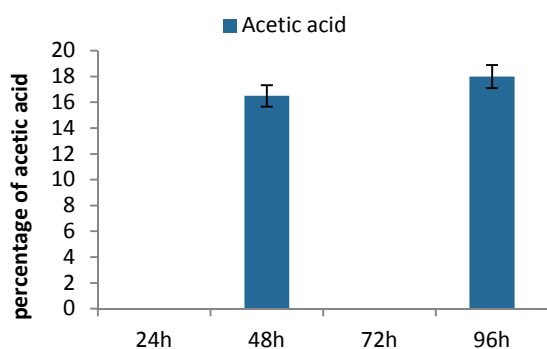
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kefir extracts were tested on wound healing on burn induced injury on rat skin.

### Materials and Methods

Kefir grains (50 g) were continuously cultured in 100 g/l of Man, Rogosa and Sharpe (MRS) Broth medium at 35 °C for 48 and 96 h in a CO<sub>2</sub> incubator. The supernatants of culture fermentation were centrifuged at 6000 rpm for 20 min at about 15°C. Then, they were filtered through a 0.22-micron millipore filter and named as kefir 48 h and kefir 96 h. In order to ensure the sterility of kefir extracts, it was necessary to culture them on Mueller-Hinton Agar at 37 °C for 24 h (17). Moreover, the pH of supernatants was measured by digital pH meter.

The percentages of citric and acetic acids in different kefir extracts were measured by the reverse phase high performance liquid chromatography assay using C18 column, UV detection wavelength: 254 nm, mobile phase of deionized water, flow: 1; then compared to the 1, 5 and 10 percent lactic acid and acetic acid (Figure 1).



**Figure 1.** The percentage of acetic acid produced by kefir fermentation at different times

The antimicrobial susceptibility was evaluated by the broth micro dilution method as previously described by the NCCLS (15). The minimum inhibitory concentration (MIC) was defined as the lowest antimicrobial concentration able to completely inhibit bacterial growth up to 24 h. MIC parameters were determined in triplicates using 0.1 ml of suspensions of *Pseudomonas aeruginosa* bacteria (standard 27853 ATCC) and sample from patients with burns (3 CFU/ml × 10<sup>8</sup> CFU/ml) in tubes containing 10 mL of brain heart infusion (BHI) solution and the same amount of kefir. Tubes were mixed using a vortex for 60 s and incubated at 37°C for 24 h. minimum bactericidal concentration (MBC) values were obtained based on the results for MIC values. Plates containing 25 ml of BHI agar medium were inoculated with 0.1 ml of the tubes showing no growth and incubated for 24 and 48 h at 37 °C.

Kefir grains (50 g) were continuously cultured in 100 g/l of MRS broth medium for 48 h, 96 h. The supernatants of culture fermentation were centrifuged, filtered and named as kefir 48 h, kefir 96

h. kefir gels products were prepared from above extract named as kefir 48 h gel, kefir 96 h gel (17,18). The kefir gels were formulated by addition of 100 ml of extract 100-100 g gel base.

Forty male Wistar rats, aged 6 months old weighting 200 ± 10 g were purchased from pastor Institute Karaj city, I.R. Iran. The rats were caged under controlled conditions of light, room temperature and humidity for a week prior to study. This study was approved by the Ethical Committee of Islamic Azad University, Tehran, Iran.

The 3<sup>rd</sup> degree burn wounds were induced on shaved area of dorsal skin of the rats under anesthesia (intraperitoneal injection of 100/5 mg/kg ketamin/xylazin) using hot plate sized 3 ± 1 cm at temperature of 156.8 F or 69.8 °C for 3 s (19). The rats were placed in an isolated cage to inhibit transmission of infection. The wounds were examined after 24 h and in case of necrotic tissue, the same was removed. Debridement procedure under the standard way was done for all the animals.

Twenty-four hour after rats were caged individually and divided into five groups of eight rats each as follows:

- Untreated group: the burn wounds received no medication
- Base gel group: the base gel was applied on burn wounds
- Silver sulfadiazine group: the silver sulfadiazine 1% was applied on burn wounds
- Kefir 48 h gel group: the kefir 48 h gel was applied on burn wounds
- Kefir 96 h gel group: the kefir 96 h gel was applied on burn wounds.

The gel and silver sulfadiazine thin layer were applied on burn wounds twice daily.

Wound area diameters were evaluated and measured by naked eyes on base line, 1 and 2 week's interval using planimetry procedure (20). In brief wounds area were calculated by manually counting squares completely or half or more within the wound border using 1 mm<sup>2</sup> designed transparent graph paper. The initial wounds size using hot plate sized 3 cm × 1 cm were 300 mm<sup>2</sup> or 100%. The percentage of wound size and recovery was calculated according to Equations (1) and (2).

$$\text{Percentage wound area} = \frac{\text{Wound area on day X}}{\text{Wound area on base line (300 mm}^2\text{)}}$$

x is the day when the wound area is measured

Percent of wound recovery = 100% of wound area (2)

The data were analyzed using SPSS for Windows (version 10.0, SPSS Inc., Chicago, IL, USA) using analysis of variance and Duncan mean comparison test. P < 0.050 was considered to be statistically significant

## Results

### Percentage of organic acids and pH

The results showed that by increasing the time of

fermentation, concentration of lactic and acetic acids in orders of Kefir 96 h > kefir 48 h increased, but acetic acid was not produced in kefir extract 24 h (Figure 1). Further, acid-producing activity of this product revealed that, pH decreased significantly ( $P \leq 0.001$ ) after 96 h compared with 48 h in Kefir extracts. It was indicated that the lowest pH was related to the 96 h fermented Kefir extract and this extract produced the highest amount of lactic and acetic acid (Figure 1).

#### Measurement of MIC and MBC

MIC and MBC for kefir extracts of 48 h and 96 h were 250 mg/ml.

#### Gross morphology examination

Results indicated that percentage of wound size were 3 cm on base line day for each rat in all the groups. At the end of the 1<sup>st</sup> week, the percentage of wound size were significantly lower in kefir 96 h gel ( $P < 0.010$ ) when compared to base gel and untreated groups as well as silver sulfadiazine treated group. At the end of the 2<sup>nd</sup> week, the percentage of wound size were significantly lower in kefir 96 h gel ( $P < 0.010$ ), kefir 48 h gel ( $P < 0.001$ ) and silver sulfadiazine 1% ( $P < 0.050$ ) when compared to base gel and untreated groups (Table 1).

#### Discussion

The results in this study showed that kefir extract as a probiotic has the ability to inhibit the activity of *P. aeruginosa* in samples taken from patients with burns and standard sample. It was found that, the diameter of inhibition zones have been increased linearly with increasing the time of fermentation. Hence, continuously cultured kefir grains in MRS broth medium up to 96 h increases these properties of extract. These results are parallel to our previous findings showing the wound healing and antimicrobial effects of Kefir gels in burn wounds infected with *P. aeruginosa* in rats. In that study great reduction in percentage of wound size in kefir grains 96 h gel and also wound healing time was significantly shorter in kefir 96 h gel among different groups of fermentation (20). Furthermore, in several in vivo and in vitro studies, kefir possesses antimicrobial activity against a wide variety of Gram-positive, Gram-negative bacteria

and some yeasts (21-23). Kefir extracts inhibit microbial growth through various mechanisms, maybe in part by the antagonistic action of various microorganisms present in kefir, which are also capable to prevent the normal action of pathogenic microorganisms (24). Kefir extracts contained several natural antimicrobials and inhibitory substances such as lactic acid, acetic acid, hydrogen peroxide, bacteriocins, reuterin, and reutericyclin that may effect on pathogens (25). Lactic acid penetrates the microbial cell membrane, causing the acidification of the cytoplasm and the enzyme activity inhibition and at a higher intracellular pH the acids dissociate to produce hydrogen ions, which interfere with important metabolic functions such as oxidative phosphorylation, a possible explanation for the inhibition of aerobic species. In addition, was found that lactic and acetic acid have an excellent synergistic inhibitory effect when produced together in Kefir extract and this effect is associated to the potentiation of acetic acid at the lower pH produced by lactic acid. Another study demonstrated that microorganisms isolated from the Kefir grains inhibited the growth of *Staphylococcus aureus* and *P. aeruginosa*. The authors suggested that organic acids, hydrogen peroxide and other substances were responsible for the inhibition (26).

In this study, by increasing the time of fermentation, concentration of lactic and acetic acids increased in orders of Kefir 96 h > Kefir 48 h > Kefir 24 h and this resulted in continuous increasing of the diameter of inhibition zone and decreasing the density of bacteria following inhibition of *P. aeruginosa* growth. In the present study, the wound healing activity and antimicrobial effects of kefir gels were tested in experimental. The antimicrobial activity of kefir 96 h gel was similar to silver sulfadiazine 1% ointment but wound healing time were lower in kefir 96 h gel when compared to silver sulfadiazine ointment. Furthermore the process of burn wound healing took place within 14 days for kefir gel 96 h in our study, but it was for 24 days for silver sulfadiazine 1% reported in previous study. These data indicated that, continuously cultured kefir grains in MRS broth medium up to 96 h increases the wound healing properties of extract.

**Table 1.** Percentage of wound size after burn wounds induction at 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days of treatment in 5 groups of 8 rats each (mean  $\pm$  SD)

Size of burn wound			
Groups	1 <sup>st</sup> day	7 days	14 days
Kefir 48 h gel	95 $\pm$ 7.1	81.8-4.6	74.6-4.2
Kefir 96 h gel	95 $\pm$ 7.1	54.6-2.8**	15.1-2.1***
untreated	95 $\pm$ 7.1	91.0-6.2	89.6-4.1
Silver sulfadiazine	95 $\pm$ 7.1	92.5-7.4	65.0-5.2*
Base gel	95 $\pm$ 7.1	96.5-3.5	92.5-3.5

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; Percentage of wound size in all treated groups were compared to untreated and base gel groups; Percentage of wound size in kefir; 96 h gel was significantly lowest at the end of 2<sup>nd</sup> week as compared to all other groups; SD: Standard deviation

The antimicrobial properties of kefir were reported on several microorganisms in laboratories as well as in human diarrheas disease and urinary tract infection. Several mechanisms were reported for antimicrobial effects of kefir grains. Farnworth reported that the antimicrobial effects of kefir grains are due to lactic acid and antibiotics produced by microorganisms. Schillinger (27) proposed that, bacteriocin and lactic acids from *Lactobacillus rhamnosus* isolated from kefir grains are responsible for such antimicrobial effects. However several other mechanisms such as production of organic acids, ethanol, bacteriocines and hydrogen peroxide, in fermented process were proposed for antimicrobial activity kefir extracts (26). The anti-inflammatory property is also influence process of wound healing. Medeiros et al. (28) reported that the positive effects of hyaluronic acid on burn injuries are due to its anti-inflammatory effects. The anti-inflammatory properties of polysaccharide present in kefir extract may also influence in process of wound healing (14,29). However the lactic acid, acetic acid, polysaccharide and other chemicals present in kefir preparation are important factors for antimicrobial, anti-inflammatory and wound healing properties observe in present study.

However in future studies, we try to standardize kefir gel product by determination of lactic acid, acetic acid and polysaccharide concentration along with its burn wounds healing properties in animal studies.

### Conclusion

The kefir gel therapy especially kefir 96 h gel with longer culture fermentation time strongly improves clinical outcomes after thermal injury when compared to conventional silver sulfadiazine treatment.

### Ethical issues

Since this study involved animal and not humans as subject of study, ethical issue is not necessary.

### Conflict of interests

We declare that we have no conflict of interests.

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