Protective properties of kefir on burn wounds of mice that were infected with S. aureus, P. aeruginosa and E. coli

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Abstract: Burns and burn wounds are very sensitive to infections and cause a large amount of death worldwide. Although burn wound is sterile at the beginning, because of the risk factors such as prolonged hospital stay, immune suppression and burn affecting large surface area, colonisation with Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli occur. For the burn therapy, one of the most important ways is to control bacterial infections. A probiotic fermented milk product kefir has antioxidant, antimicrobial, antinflammatory, anticancer and various health promoting features. This study aims to examine possible protective properties of kefir which was used on the burn wounds that were infected with S. aureus, P. aeruginosa and E. coli. Swiss albino / Balb-c mice were seperated before into four groups: (1) used as control group, (2) second-degree burn model+ burn wounds were infected with Paeruginosa + S.aureus + E.coli, (3) second-burn wounds were treated with sterile pads dressed with kefir and (4) second-degree burn+burn wounds were infected with P. aeruginosa + S.aureus +E.coli before being treated with sterile pads dressed with kefir. The serum biochemical results verified the histopathological results and our findings showed that kefir is an eective product with cell-protecting properties.

Key words: Second-degree burn; Kefir; Wound healing; Infections; S.aureus; Paeruginosa; E.coli; Mice.

Introduction

Burns and burn wounds are one of the health issues in modern life due to their serious damages to the patients and to the family relationships (1). Burn area infection is a prominent problem in burn treatment and is the most common factor of fatality after burns (2,3). The microorganisms causing burn infection may be infected from the environment during the treatment of patients in burn units or from another patient treated in the same unit (4,5). Burn wound infections by Pseudomonas aeruginos, Escherchia coli and Staphylococcus aureus are more common causes of mortality (2,6). S.aureus that is gram-positive is the a major reason of burn wound infections (3) and commonly end up with septicemia (7,8). In the gram-positive bacteri infected burns hyperthermia, leukocytosis, behavioral disorders, mental confusion are usually seen and around of the wound cellulitis and exuded maceration occur. Hypothermia and leukopenia are common in burn infections caused by gram negative bacteria. And the patients may be confused (4).

Alternative medicines with natural products are cheaper options and becoming increasingly common (9,10). Probiotic products are reported to strengthen the immune system, reduce wound healing process and inflammation following lymphocyte accumulations in wound area (11,12). Probiotics are being used for allergic diseases, bacterial vaginosis, urinary and gastrointestinal system infections (13). Probiotic microorganisms can inhibit the adverse effect of pathogens, strengthen the immune response and intestinal barrier (14,15). Potential health research has focused on fermented milk from cows, ewes and goats milk such as kefir which is well known as a major source of probiotic (16-18). Kefir is a famous fermented product with its potential health benefit properties that arise from the microbial species that kefir grains have which are also associated with kefir fermentation (19). Kefir grains include a rich microbial community formed by bacterial and yeast microflora responsible for the kefir fermentation (20,21). Kefir contains rich health-benefiting properties, exhibits antioxidative, antimicrobial, anticarcinogenic and different health supporting activity (22,23). Kefir addition has diverse health benefits such as gastro-intestinal cell proliferation, antibacterial, anti-mutagenic, anti-inflammatory, antiallergic, hypocholesterolemic, anticarcinogenic, and 

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Materials and Methods

Kefir Fermentation
In this study, freeze-dried kefir culture was commercially purchased from Faculty of Agriculture, Dairy Product Technology, Ege University, Turkey to produce kefir. This lightly natural aromatic kefir grains with 1% pasteurized cow milk were preferred for kefir fermentation. The method used for kefir fermentation was reported by Marshall et al. fermented kefir was incubated for 24h at the room temperature (24-26 °C) (26). At the end of the fermentation period, kefir was cooled to about +4 °C and kept at this temperature for usage. The kefir products after fermentation were centrifuged. Supernatant portions were taken and used in the study. Kefir was matured at 4 °C for at least one day before use and fermentation was renewed daily and used fresh.

Preparation of Bacterial Cultures and contamination of the burn wounds
*Escherichia coli* (ATCC23276), *Staphylococcus aureus* (ATCC26542) and *Pseudomonas aeruginosa* (ATCC 25338) control strains were commercially purchased from SACEM Hayat Teknolojiler A.S., Turkey to use in the study. Blood Agar Base was used for *Staphylococcus aureus* (ATCC26542). The Selective Agar Base was used for *Pseudomonas aeruginosa* (ATCC 25338). Eosin Methylene Blue agar was used for *Escherichia coli* (ATCC23276). The three bacteria were all diluted to 1.5x10^8 CFU.mL^-1 with 0.9% sodium chloride solution and the experiment was repeated three times to determine the minimum inhibitory concentration (MIC).

Antimicrobial determination of kefir in vitro
In order to test the MIC parameters of the kefir, kefir which were kept for 24 h were added to the tubes containing 10 ml Müller Hinton Broth (MHB). Then samples were prepared containing 0.1 ml of bacterial suspension (3x10^8 CFU / ml). The mixture was stirred using vortex for 60 seconds, then, incubated at 37 °C for 24 h. MIC levels were obtained. Then, MHA medium was sown, and no growth samples were included in the study (27). MIC values of kefir for *S.aureus* were 2.42 mg·mL^-1, *P.aeruginosa* 7.9 mg·mL^-1, and *E.coli* were 4.55 mg·mL^-1.

Animals and burn model preparation
*Swiss albino* / *Balb-c* mice were provided from SACEM Hayat Teknolojiler A.S., Turkey to produce kefir. This lightly natural aromatic kefir grains with 1% pasteurized cow milk were preferred for kefir fermentation. The method used for kefir fermentation was reported by Marshall et al. fermented kefir was incubated for 24h at the room temperature (24-26 °C) (26). At the end of the fermentation period, kefir was cooled to about +4 °C and kept at this temperature for usage. The kefir products after fermentation were centrifuged. Supernatant portions were taken and used in the study. Kefir was matured at 4 °C for at least one day before use and fermentation was renewed daily and used fresh.

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time (29). Burn injuries are so traumatic and physically debilitating that these can adversely affect almost all the organs (30). Because of epidermis lost after burn injury, the epidermis becomes susceptible to infections and this causes significant morbidity and mortality in burn trauma cases. As a matter of fact; from our findings in activity, exhaustion, trembling and weakness were seen in the burn + bacteria infected 2nd group when we compared to the other groups. Burn wounds can be treated with different methods depending on the severity of the burns. Researches (31,32) demonstrated that topical antibiotics treatment is mostly used but due to its adverse effects such as bacterial resistance and insufficient on wound healing process, search for alternative natural products is a growing interest. It was observed from our findings that mice in kefir treated burn group are seen more mobile and healthy when compared to burn + infection group.

Tissue inflammation occurs after exposure to thermal heat in the tissues of the burn stasis region and tissue edema is seen. During this inflammation, cytotoxic cytokines and reactive oxygen species (ROS) are released from the neutrophils collected in the medium. Due to prolonged inflammation, burned cytokines and ROS are not able to compensate antioxidant mechanisms, resulting in damage to vital structures such as lipid, protein and nucleic acid (33). Ma et al. (34) reported that neutropenia is one of the common complications of burn. Our result showed that leucocytes, thrombocytes, neutrophil, monocyte and lymphocyte numbers were increased in the 2nd group (Table 1). This is a sign of severe tissue damage and inflammation in the burn area. Therefore, it was important to search for more efficient agents with fewer adverse effects for healing of burns. Lopitz et al. (35) reported that kefir is found to act against pathogenic bacteria. Also it was demonstrated that kefir can stimulate innate immune responses in defense against pathogens (36,37). According to our results, the number of neutrophil, leucocytes, thrombocytes, monocyte and lymphocyte is lower in the kefir given 3rd group than all the other groups despite of the burn injury (Table 1). Therefore, we may infer that topical application of kefir can concurrently protect burn wounds from infection and accelerate the healing process.

Kefir can be used as topical treatment for burn injury for its anti-microbial activity. Our findings showed that kefir dressing healed the burn wound and reduced the burn wound area increasingly (Fig. 1). We may infer from this; for burn treatment, the important way is to control the bacterial infection. Parallel to our study Husseiniet al. (25) observed that the lactic acid, acetic acid, polysaccharides and other chemicals present in kefir are important for wound healing properties and wounds are importantly lower in kefir compared to control, base gel and silver sulfadiazine dressing groups.

![Figure 1](image-url) Figure 1. The appearance of the 2nd, 3rd and 4th Groups’ burn wounds with eschar on last days of study before dissection.

### Table 1. The number of leucocytes, thrombocytes, neutrophil, monocyte and lymphocyte. Kruskal Wallis One Way Analysis of Variance on Ranks. (Median (25%-75%)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±Std. Deviation</th>
<th>Median (25%-75%)</th>
<th>P</th>
<th>Multiple Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (Control)</td>
<td>11,36±1,91</td>
<td>10,60 (9,95-12,99)</td>
<td>0,001</td>
<td>1-2</td>
</tr>
<tr>
<td>Group 2</td>
<td>19,67±2,34</td>
<td>20,00 (17,50-22,00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>14,20±2,06</td>
<td>14,10 (12,65-16,20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>16,37±1,99</td>
<td>16,60 (14,15-18,20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (Control)</td>
<td>275,33±34,79</td>
<td>277,00 (250,00-303,50)</td>
<td>0,004</td>
<td>1-2, 1-3, 1-4</td>
</tr>
<tr>
<td>Group 2</td>
<td>403,3±40,69</td>
<td>399,00 (367,50-428,00)</td>
<td></td>
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<tr>
<td>Group 3</td>
<td>390,67±49,78</td>
<td>396,00 (342,50-425,50)</td>
<td></td>
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<tr>
<td>Group 4</td>
<td>401,00±58,06</td>
<td>385,00 (336,00-431,00)</td>
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<tr>
<td>Thrombocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>26,67±3,27</td>
<td>27,00 (23,50-30,00)</td>
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</tr>
<tr>
<td>Group 3</td>
<td>31,60±15,65</td>
<td>37,50 (25,80-40,05)</td>
<td>0,012</td>
<td>1-2, 1-4</td>
</tr>
<tr>
<td>Group 4</td>
<td>38,37±1,42</td>
<td>38,50 (37,50-39,30)</td>
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<tr>
<td>Group 1 (Control)</td>
<td>38,97±6,11</td>
<td>39,40 (32,60-44,65)</td>
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<tr>
<td>Neutrophils</td>
<td></td>
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<tr>
<td>Group 2</td>
<td>2,07±0,33</td>
<td>2,10 (1,75-2,40)</td>
<td>0,001</td>
<td>1-2</td>
</tr>
<tr>
<td>Group 3</td>
<td>3,07±0,35</td>
<td>3,10 (2,75-3,30)</td>
<td></td>
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</tr>
<tr>
<td>Group 4</td>
<td>3,20±0,40</td>
<td>3,15 (2,80-3,58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (Control)</td>
<td>70,67±6,02</td>
<td>69,00 (65,50-78,00)</td>
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<tr>
<td>Group 2</td>
<td>81,67±8,04</td>
<td>85,00 (74,00-88,00)</td>
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<tr>
<td>Monocytes</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>79,00±4,86</td>
<td>80,00 (76,00-82,50)</td>
<td>0,076</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Group 4</td>
<td>80,33±9,16</td>
<td>83,00 (73,00-88,00)</td>
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<tr>
<td>Lymphocytes</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>80,00±4,86</td>
<td>80,00 (76,00-82,50)</td>
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<td>83,00 (73,00-88,00)</td>
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</table>
Inflammatory and antimicrobial effect of the kefir at the cell level. As a matter of fact, in the 3rd group it was seen that kefir could protect tissue cells in spite of the infected burn wound (Fig. 2).

At the end of the experiment, wound tissues were removed from the mice for histopathologic examination. The results confirmed that skin samples with burn wounds were exposed to more severe infiltration of inflammatory cells and necrosis of the epidermis and hypodermis compared to normal skin tissues. The tissues taken from kefir treated 3rd group were seen as a thick and well developed epidermal layer similar to normal skin. Additionally, inflammatory cells infiltrates was less than control. Histological findings showed that kefir may support epidermal regeneration and accelerate wound healing (Fig. 2, Table 2). Epithelization, collogenization and eschar reduction were found to happen in much shorter time in the kefir administered 3rd group to compared with burn+infection 2nd group and control (Table 2). Also, based on Table 2 findings, we can indicate that kefir dressing importantly accelerates angiogenesis in burn tissue. Likewise, it is observed that kefir had a positive effect on fibroblast proliferation and collogenization during burn wound healing (Table 2). Huseini et al. (25) reported that epithelization and scar formation were markedly higher while inflammation were markedly less in kefir treated group compared to silver sulfadiazine and control (1st) groups.

Although inflammation is an significant situation in the wound healing, it can also inhibit the healing process (38). Shupp et al. (33) explored the prominent inflammatory cell infiltration of burn wound progression and proposed that after burn injury, the burn wound experiences a prolonged inflammatory response in which neutrophils release cytotoxic cytokines and ROS. Moreover, persistent neutrophil aggregation in postcapillary venules contributes to vascular occlusion and edema. As a matter of fact, in present study, inflammatory cell infiltration increased in the 2nd group compared to the other groups.

Based on our findings, we can say that kefir dressing accelerates angiogenesis and vascular proliferation in mice burn skin (Table 2). Similar to our study, Zhang et al. showed that SOP dressing on burn wound decreases wound contraction and epithelization process and supports collogenization compared to control (28).

From the findings of this study we may infer that kefir could accelerate the healing of second-degree burns with its potent antibacterial property. Similarly Huseini et al. showed that kefir has better wound-healing activity than conventional silver sulfadiazine treatment (25). A study by Kamila et al. showed that kefir has a better wound healing activity comparing to the clostebol-neomycin treatment (39). Also, Rodrigues et al. reported that rats treated with kefir, showed a faster healing activity comparing to clostebol-neomycin treatment on infected-wound (40).

From the findings on Table 3 it was seen that no important body weight changes were observed in all the groups at the end of the experiment. However, it was observed that the body weight decreased in the 2nd and 4th groups, especially in the 2nd group. In the 3rd group mice gained weight, this can be a result of kefir (Table 3).

There cover signs in the results of biochemical findings were verified the histopathological results. Our results indicate that kefir is an effective burn wound healing product with cell and tissue protecting activities. Kefir which is a promising treatment for the second-degree burns is an important antimicrobial. According to the results, it could be concluded from our findings that kefir accelerates the healing of second degree burn wound and prevents infection inflammationally. We may infer that kefir is a good candidate for a therapeutic product for burn treatment and has an effective antibacterial activity against burns but in-depth more studies will be needed to evaluate its clinical application on humans. Furthermore, considering the lack of information on the

### Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight before treatment (gr)</th>
<th>Body weight after treatment (gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>26.42</td>
<td>31.25</td>
</tr>
<tr>
<td>Group 2</td>
<td>28.15</td>
<td>23.42</td>
</tr>
<tr>
<td>Group 3</td>
<td>27.24</td>
<td>29.38</td>
</tr>
<tr>
<td>Group 4</td>
<td>28.76</td>
<td>25.61</td>
</tr>
</tbody>
</table>

**Figure 2.** Haematoxylin and eosin (H&E) staining of skin tissue. A-Microscopic images of control group, B-Microscopic images of burn + infected 2nd group, C-Microscopic images of burn + kefir treated 3rd, D-Microscopic images of burn + infection + kefir treated 4th group.

**Table 3.** The body weight of mice after kefir treatment at the end of experiment. Data are expressed as mean ± S.D.
effect of kefir on the development of burn wound, these results can provide a preliminary platform for future researches.

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Author contributions

Conflicts of interest
There are no known conflicts of interest associated with this publication. We confirm that the manuscript has been read and approved by all named author.

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