Original Article

The effectiveness of kefir in acute renal failure due to glycerol-induced rhabdomyolysis

Pinar Karabacak¹, Filiz Alkaya Solmaz¹, Fatih Gultekin², Meral Oncu³, Ozlem Yuksel², Meltem Ozgoçmen³, Ilter Ilhan²

Departments of ¹Anesthesia and Reanimation, ²Medical Biochemistry, ³Histology and Embryology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

Received March 9, 2016; Accepted August 13, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: Acute renal failure (ARF) is one of the important complications of rhabdomyolysis. Kefir is an antioxidant, antineoplastic and it lowers cholesterol levels and protects from infections. We evaluated the efficiency of kefir on rats with renal failure after rhabdomyolysis. Each weight 200-250 grams, 4 groups of Wistar Albino female rats which contain 8 rats per group are used. Biochemical parameters such as renal functional tests, Cystatin C, total antioxidant capacity (TAC), total oxidative stress (TOS), laktat dehydrogenase (LDH) and creatine phosphokinase (CPK) are studied. Additionally, renal necrosis degree was evaluated with histopathological analysis. We observed nephropathy and rhabdomyolysis in rhabdomyolysis group and kefir + rhabdomyolysis group. Cystatine C and LDH were significantly higher in rhabdomyolysis group (P < 0.01). When all the groups were evaluated, urea (P < 0.01), creatinine (P < 0.01), Cystatine C (P < 0.01), LDH (P < 0.01), TOS (P = 0.02) and CPK (P = 0.03) levels were significantly higher. The histopathological comparison of groups; degree of necrosis is lower in the kefir + rhabdomyolysis group than in the rhabdomyolysis group. But biochemically there were no differences within these two groups. Biochemically and histopathological comparison of groups there were no differences within other two groups. We showed that kefir has a histopathological preventive attribute on rats with rhabdomyolysis-induced ARF.

Keywords: Kefir, acute renal failure, rhabdomyolysis, cystatine C

Introduction

Acute renal failure (ARF) is common in intensive care unit patients and its prevalence is gradually increasing. Morbidity and mortality rates associated with ARF remain high despite advancements in treatment [1]. Rhabdomyolysis is a syndrome that develops as a result of skeletal muscle structure and myoglobin impairment, and passage of intracellular protein and electrolytes into circulation. Iron and iron-containing proteins (myoglobin and hemoglobin) produced via myolysis and hemolysis lead to free radical formation, consumption of nitric oxide stores, and vasoconstriction, ultimately leading to impaired renal function [2-4]. ARF due to rhabdomyolysis is defined as myoglobinuric acute renal failure (MARF) [5].

Kefir is a food product made via fermentation of milk with kefir particles or culture [6]. It contains many exogenous fatty acids and amino acids necessary for the human body that should be ingested with food. Sphingomyelins found in kefir and kefir oil are reported to stimulate the immune system against infections [7]. The basic function of the microorganisms in kefir is to produce lactic acid, antibiotic, and anti-bactericidals. Many lactic acid bacteria have systems that metabolize oxygen radicals. Stecchini et al. reported that superoxide dismutase and high magnesium content are the most important antioxidant systems [8]. Regular consumption of 500 mL of daily milk was shown to positively affect hepatic, biliary, renal function, and blood circulation, in addition to having a metabolism-stabilizing effect [7].

The present study aimed to investigate the antioxidant effect of kefir on renal tissue and function in a rat model of MARF experimentally induced using hypertonic glycerol, which is considered equal to that in humans [2].
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Methodology

Preparation of animals

The study included 32 female rats aged 10-12 weeks and weighted 200-250 g that were obtained from the Süleyman Demirel University Test Animals Laboratory. The animals were housed under standard humidity, light (12 h daylight/12 h night), and temperature (23°C) conditions, and were fed standard rat feed and water for 30 days. Anesthesia was administered on 31st day as intraperitoneal ketamine 80 mg kg⁻¹ + xylazine 10 mg kg⁻¹. The abdomen of each rat was opened using a midline incision after anesthesia and blood samples were obtained from the abdominal aorta. The Süleyman Demirel University Animal Usage Regulation Committee and Ethics Committee (3209-TU-2) approved the study protocol.

Experimental design

The rats were divided into 4 groups of 8 rats. Group 1 (control group) rats were fed standard rat feed and water for 30 days, rhabdomyolysis was not induced. Group 2 (rhabdomyolysis group) rats were fed standard rat feed and water for 30 days. Then, rhabdomyolysis was induced via administration of 50% glycerol 9 mL kg⁻¹ as 1 mL to the muscle tissue in each leg following subcutaneous administration of morphine 15 mg kg⁻¹ (approximately 0.4 mL). Group 3 (kefir group) rats were fed with standard rat feed and water plus 2 × 1 mL of kefir (4-8 mL•kg⁻¹•d⁻¹) for 30 days, rhabdomyolysis was not induced. Group 4 (kefir + rhabdomyolysis group) rats were fed with standard rat feed and water plus 2 × 1 mL of kefir (4-8 mL•kg⁻¹•d⁻¹) for 30 days. Then, rhabdomyolysis was induced via administration of 50% glycerol 9 mL kg⁻¹ as 1 mL to the muscle tissue in each leg following subcutaneous administration of morphine 15 mg kg⁻¹ (approximately 0.4 mL).

Blood sample analysis

Blood samples were centrifuged at 5000 rpm for 8 minutes. Separated serum samples were stored at -80°C until analyzed. Urea, creatinine, blood urea nitrogen (BUN), and creatine phosphokinase (CPK)/laktat dehydrogenase (LDH) were measured using commercial kits and a Beckman Coulter Au 5800 (USA) autoanalyzer in order to evaluate renal function and rhabdomyolysis. Cystatin C was measured via ELISA using rat-specific kits (Biovendar, Czech Republic) in order to evaluate renal function.

Histological examination

Anesthesia was administered on 31st day, the abdomen of each rat was opened using a midline incision after anesthesia was achieved and the rats’ kidneys were removed under sterile conditions and then washed with saline solution. The right kidney of each rat was used for histopathological examination and the left kidney was used for biochemical analysis. In order to evaluate kidney oxidant and antioxidant activity, microprotein levels in kidney tissue buffered in phosphate were studied via spectrophotometry using a Beckman Coulter Au 5800 (USA) autoanalyzer. Total antioxidant capacity (TAC) and total oxidant status (TOS) were determined using rat specific Rel Assay Diagnostics assay kits, a Beckman Coulter Au 5800 (USA) autoanalyzer, and spectrophotometry; results were calculated via division by the microprotein level. TAC and TOS were measured using the method developed by Erel [9].

Preparations were stained with hematoxylin & eosin (routine staining method) and analyzed using an Olympus BX50 binocular light microscope at 100 ×, 200 ×, and 400 × magnification; microphotographs were obtained and evaluated. A scoring system was used to determine the level of renal injury, according to the necrosed cell ratio in the cortical proximal tubular segment, as follows: 0: no necrotic cells; 1: necrotic cell count < 10%; 2: necrotic cell count 10%-25%; 3: necrotic cell count 25%-50%; 4: necrotic cell count > 50%.

Statistical analysis

A statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) 11.0 package program. Continuous variables are shown as mean ± standard deviation (SD) and categorical variables as percentage. The Kruskal-Wallis test was used for biochemical comparison of the 4 groups, and the level of statistical significance was set at P < 0.05. The Mann-Whitney U test with Bonferroni correction was used for inter-group comparisons when significant differences were noted; P < 0.01 was considered statistically significant when Bonferroni correction was performed.
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A comparison of laboratory parameters and weight between groups is shown in Table 1. In total, 3 rats in rhabdomyolysis group and 3 rats in kefir + rhabdomyolysis group were excluded from the study because nephropathy was not observed biochemically; the study was completed with 26 rats. There wasn’t a significant difference in baseline weight (weight 0) between groups (P = 0.27); however, there was significant difference among control group, kefir group, and kefir + rhabdomyolysis group with a 30-day feeding (P = 0.04). When all the groups were evaluated together there was a significant difference in urea (P < 0.01), BUN (P < 0.01), creatinine (P < 0.01), cystatin C (P < 0.01), LDH (P < 0.01), CPK (P = 0.03), and TOS levels (P = 0.02) between the groups, whereas TAC levels were similar (P = 0.49). However, when the groups were evaluated individually; urea, BUN, creatinine, cystatin C and LDH values were similar between control group and kefir group, between rhabdomyolysis group and kefir + rhabdomyolysis group, but significantly different between other groups (Table 1, P < 0.01). The CPK level was similar between groups (P > 0.01). There wasn’t a significant difference in TAC and TOS values between the groups (Table 1, P > 0.01).

Histological analysis of renal tissue sections of the rats in control group and kefir group showed no pathologic findings (Figures 1 and 2). Histopathological analysis of renal tissues in rhabdomyolysis group showed vascular congestion, inflammatory cell infiltration, tubular dilation, and hydropic degeneration in tubular epithelial cells (Figure 3). Pathologic findings in renal tissues in kefir + rhabdomyolysis group improved after the rats were given kefir (Figure 4) (P < 0.05). Histologic (structural) evaluation

| Table 1. Comparison of weight and laboratory characteristics between groups |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Variable                    | Group I (n = 8)              | Group II (n = 5)             | Group III (n = 8)            | Group IV (n = 5)             |
| Weight beginning, mg        | 238±14                      | 224±20                      | 231±21                      | 221±21                      |
| Weight 1st month, mg        | 234±14★,€                   | 208±21                      | 226±26                      | 206±20                      |
| Urea, mg/dl                 | 50±5★,€                     | 191±85¥                     | 51±7€                       | 181±77                      |
| BUN, mg/dl                  | 23±3★,€                     | 90±40¥                      | 24±3€                       | 85±36                       |
| Creatinine, mg/dl           | 0.46±0.03★,€                | 1.83±0.89¥                  | 0.45±0.04€                  | 1.75±0.80                   |
| Cystatin C, mg/L            | 1.56±0.32★,€                | 5.42±3.04¥                  | 1.32±0.56€                  | 4.26±2.18                   |
| LDH, U/L                    | 465±245★                    | 1456±1143¥                  | 124±68€                     | 1148±1077                   |
| CPK, U/L                    | 215±127                     | 525±540                     | 137±172                     | 250±178                     |
| TAC, Trolox equivalent/L    | 2.50±0.19                   | 2.34±0.19                   | 2.70±0.63                   | 2.37±0.38                   |
| TOS, Trolox equivalent/L    | 87±8                        | 101±23                      | 80±10                       | 139±50                      |

BUN: blood urea nitrogen, CPK: creatine kinase, LDH: lactate dehydrogenase, TAC: total antioxidant capacity, TOS: total oxidative stress. Values are given as mean ± standard deviation or number. P Values represent the comparisons among four groups.

Group I: control, Group II: rhabdomyolysis, Group III: Kefir, Group IV: Kefir and rhabdomyolysis Group.
★: P < 0.01 vs. group II, €: P < 0.01 vs. group IV, ¥: P < 0.01 vs. group III.

Figure 1. Histopathological kidney section of the control group (group I). Normal histological findings. Kidney corpusculum (thin arrow), tubules (thick arrow), (Hematoxylin-eosin, × 10).

Figure 2. Histopathological kidney section of Kefir group (group III). Normal histological findings. Medulla area, collector tubules (arrows), (Hematoxylin-eosin, × 10).

Results

A comparison of laboratory parameters and weight between groups is shown in Table 1. In total, 3 rats in rhabdomyolysis group and 3 rats in kefir + rhabdomyolysis group were excluded from the study because nephropathy was not observed biochemically; the study was completed with 26 rats. There wasn’t a significant difference in baseline weight (weight 0) between groups (P = 0.27); however, there was significant difference among control group, kefir group, and kefir + rhabdomyolysis group with a 30-day feeding (P = 0.04). When all the groups were evaluated together there was a significant difference in urea (P < 0.01), BUN (P < 0.01), creatinine (P < 0.01), cystatin C (P < 0.01), LDH (P < 0.01), CPK (P = 0.03), and TOS levels (P = 0.02) between the groups, whereas TAC levels were similar (P = 0.49). However, when the groups were evaluated individually; urea, BUN, creatinine, cystatin C and LDH values were similar between control group and kefir group, between rhabdomyolysis group and kefir + rhabdomyolysis group, but significantly different between other groups (Table 1, P < 0.01). The CPK level was similar between groups (P > 0.01). There wasn’t a significant difference in TAC and TOS values between the groups (Table 1, P > 0.01).

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Effectiveness of kefir in acute renal failure

The present findings show that kefir administered to rats that developed ARF following rhabdomyolysis induced via glycerol did not cause biochemical improvement, but improved histological findings. In TAC, TOS and Cystatin C levels in Kefir groups was not observed to improve. However, pathologic findings in rhabdomyolysis group were significantly improved after the rats were given kefir.

Acute renal failure is defined as a serum creatinine level > 50% higher than the basal level or > 0.5 mg dL\(^{-1}\). It’s associated with impaired renal function that occurs within hours-days and a reduction in the glomerular filtration rate (GFR) [1]. Its prevalence gradually increases in intensive care units. The morbidity and mortality rates associated with ARF remain high despite treatment advancements [10]. Rhabdomyolysis is a syndrome that develops as a result of myoglobin, intracellular protein, and electrolytes entering circulation. ARF that develops due to rhabdomyolysis is defined as MARF. A large amount of myoglobin released from necrotic muscle cells is freely filtrated by glomerules and reabsorbed by renal tubules, leading to direct injury. This pigment can also cause injury via distal renal tubular obstruction [5]. Traumatic muscle destruction was first defined by German Von Colmers after the Messina Earthquake [11]. Rhabdomyolysis can also occur due to trauma, excessive physical activity, seizures, alcohol and other drug use, and after infections [12]. Crush syndrome is a complicated condition consisting of trauma-induced rhabdomyolysis and associated surgical/medical signs and symptoms [13]. In 1941 Bywater and Beall reported the coexistence of skeletal muscle crush injury and acute tubuler necrosis [14]. Mortality increases in cases with crush syndrome that develops after an earthquake, and early diagnosis and treatment are crucial [13, 15].

The serum creatinine level is commonly used to diagnose ARF. Creatinine is a poor marker of acute deterioration of renal function. The serum creatinine level is affected by many extra-renal factors, including body weight, gender, age, total body volume, drug use, muscle metabolism, and protein intake. In contrast, the cysteine protease inhibitor cystatin C is not affected by age, gender, or muscle mass, is freely filtrated by glomerules and completely reabsorbed by proximal tubule cells, and is catabolized; therefore, it is much more sensitive than the serum creatinine level as marker of changes in GFR. In addition, the creatinine level elevates 48-72 h following renal injury. Measurement of the serum cystatin C level was observed to be

Figure 3. Histopathological kidney section of Rhabdomyolysis Group (group II). Inflammatory cell infiltration (asterisk), hydropic degeneration in tubules epithelial cells (thin black arrow), tubules dilatation (white arrow), vasculler congestion (thick black arrow), (Hematoxylin-eosin, × 10).

Figure 4. Histopathological kidney section of Kefir and rhabdomyolysis Group (group IV). Inflammatory cell infiltration (white arrow), hydropic degeneration in tubules epithelial cells (thin black arrow), vasculler congestion (thick black arrow), (Hematoxylin-eosin, × 10).
Table 2. Histopathological comparison of all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammatory</th>
<th>Vascular</th>
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I-II: P < 0.05  P < 0.05  P < 0.05  P < 0.05  
I-III: P > 0.05  P > 0.05  P > 0.05  P > 0.05  
I-IV: P < 0.05  P < 0.05  P < 0.05  P < 0.05  
II-III: P < 0.05  P < 0.05  P < 0.05  P < 0.05  
II-IV: P < 0.05  P < 0.05  P < 0.05  P < 0.05  
III-IV: P < 0.05  P < 0.05  P < 0.05  P < 0.05  

Group I: control, Group II: rhabdomyolysis, Group III: Kefir, Group IV: Kefir and rhabdomyolysis group. P value of < 0.05 was significant.

Effectiveness of kefir in acute renal failure

Humans have used Kefir continuously since the early 8th century due to its health promoting/healing properties. It is currently used to treat diseases caused by pathogens in many regions of the world due to the beneficial microorganisms it contains. In addition, kefir was shown to have anti-tumor and immunostimulant effects, antioxidant activity that reduces lipid peroxidation, and anti-diabetic, antibacterial, and antifungal effects in animals [6, 7]. Regular daily consumption of 500 mL of kefir was reported to stabilize metabolism, positively affect liver, gallbladder, and renal function, and blood circulation. Kefir was also reported to lower the cholesterol level [7, 17]. Based on such reports, the present study aimed to investigate the effect of kefir in rats with rhabdomyolysis-induced ARF. Rhabdomyolysis develops experimentally in rats via intramuscular IM administration of hypertonic glycerol and MARF develops secondarily. This model is also used as an experimental model of MARF in humans; therefore, glycerol was used to induce rhabdomyolysis in the present study. Regional myolysis, hemolysis, and intravascular volume decrease in response to intramuscular injection of hypertonic glycerol [3, 12].

The iron ion that is produced as a result of degradation of myoglobin and hemoglobin catalyzes Haber Weiss and Fenton reactions, which results in radical formation, lipid peroxidation, and impaired renal function [18, 19]. Recent studies have shown that free radicals play an important role in the pathogenesis of renal injury of ischemic and toxic origin [20]. In addition, clinical and experimental studies reported that various antioxidant agents were effective when used together with conventional ARF treatments [4, 21]. Brivet et al. reported that nutritional status was a factor affecting the prognosis in patients with ARF [10]. Dietary intake of antioxidants was shown to elevate antioxidant enzyme levels in animal studies [22]. Kefir a known potent antioxidant is considered to be beneficial for health in countries where it is a part of nutrition culture; however, the number of studies on the effect of kefir on animal and human nutrition is insufficient [23].

The antioxidant effect of kefir was shown in various studies [24-26]. In the present study, the antioxidant effect of kefir had a positive histopathological effect in rats with ARF. We anticipate that this effect was due to the increase in some antioxidant parameters. However, the antioxidant effect of kefir was not biochemically observed, which might be due to the dose of kefir administered to the rats. Precise data on the dose and duration of kefir administration are not available; the dose and duration of kefir administration varies by study [23, 26-28]. In our study, rats were fed kefir for 30-day, as reported by Figler and colleagues [26], and then laparotomy was performed on 31st day. The dose of kefir was 2 mL/rat in a study by Demirel and colleagues [28]. Güven and colleagues [24] reported that kefir was more protective than vitamin E via increasing glutathione and glutathione peroxidase levels and reducing lipid peroxidase in a rat model of...
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CC14-induced oxidative injury. In contrast to our study, they observed positive biochemical effects of kefir on oxidative stress, Çenesiz and colleagues showed that the antioxidant effect on abnormal crypt formation in the colon induced by azoxymethane was higher in rats given kefir [25].

Miller and colleagues developed a novel test (TAC) for measuring total antioxidant status. The most important advantage of TAC is that it measures the antioxidant capacity of all antioxidants in a biological sample, not just the antioxidant capacity of a single compound [29]. Antioxidant enzyme levels such as superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase (GPX) in ischemia-reperfusion injury dependent ARF were shown to significantly decrease in a study [30]. To the best of our knowledge no study has reported the effectiveness of kefir in rhabdomyolysis-induced acute tubular necrosis although it was shown to be a potent antioxidant in several studies. In the present study there wasn’t a significant biochemical difference between TAC and TOS values.

Limitation of the study

The present study has some limitations. Firstly, the small study population limits the statistical power of the findings. Secondly, it is not known if the lactobacilli in the kefir used in this study had antioxidant properties. Lastly, the duration of kefir administration was limited to 30 d; administration of longer duration might have yielded more useful findings.

Conclusion

Kefir administered to rats that developed ARF following glycerol-induced rhabdomyolysis did not cause biochemical improvement, but improved histological findings. The findings suggest that kefir might be beneficial in patients that develop MARF; however, the optimal dose and duration of its use remain unclear. Additional research with larger samples is needed to discern the optimal therapeutic dose and duration of kefir in cases of renal failure due to rhabdomyolysis.

Acknowledgements

This study was financially supported by the Süleyman Demirel University Scientific Research Projects Management Unit (project number: 3209-tu2). The authors wish to thank SüleymanDemirel University Scientific Research Projects Management Unit and Experimental Research Center for the supply of rats.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Pinar Karabacak, Department of Anesthesia and Reanimation, Medical Faculty, Suleyman Demirel University, Isparta, Turkey. Tel: 05056846286; Fax: +90 246 223783; E-mail: drpinara@gmail.com

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