Nobel Med 2026; 22(2): 116-124

EFFECTS OF KEFIR SUPPLEMENTATION ON BLOOD GLUCOSE AND RENAL TLR-2 AND TLR-4 GENE EXPRESSION IN DIABETIC RATS

Mustafa Kahraman[1,2,3], **Eren Andıç**[4,5], **Hasan Nizamoğlu**[4,6], **Elif Beyza Boz**[4,7], **Sükrü Öztürk**[8]

[1] *TUSEB Türkiye Institute of Public Health and Chronic Diseases, Istanbul, Türkiye*
[2] *Istanbul University, Istanbul Faculty of Medicine, Department of Physiology, Istanbul, Türkiye*
[3] *Canakkale 18 Mart University, Faculty of Medicine, Çanakkale, Türkiye*
[4] *Istanbul University, Istanbul Faculty of Medicine, Istanbul, Türkiye*
[5] *Istanbul University, Istanbul Faculty of Medicine, Department of Neurosurgery, Istanbul, Türkiye*
[6] *Istanbul University, Istanbul Faculty of Medicine, Department of General Surgery, Istanbul, Türkiye*
[7] *Gaziosmanpasa University, Faculty of Medicine, Department of Eye Diseases, Tokat, Türkiye*
[8] *Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Türkiye*

ABSTRACT

Objective: *This study aimed to investigate the effects of kefir supplementation on blood glucose regulation and renal Toll-like receptor (TLR-2 and TLR-4) expression in a rat model of diabetes, providing insights into the potential anti-inflammatory mechanisms of probiotic-fermented foods.*

Material and Method: *Thirty-five rats included in the study were randomly assigned to four groups: control (C), kefir-treated (K), diabetic (D), and kefir-treated diabetic (DK). To prevent hyperglycemia-related complications, D and DK rats received 1–3 units/day of insulin aspart according to uniform clinical criteria, while K and C groups received no insulin. Blood glucose levels of the rats were measured, after which they were sacrificed, and their kidneys were collected and stored at –80°C to preserve tissue integrity. And then rat kidneys were analyzed. RNA was extracted and reverse-transcribed to cDNA. Quantitative real-time PCR was performed to assess TLR-2 and TLR-4 expression.*

Results: *In diabetic rats, blood glucose levels were significantly elevated compared to the control group (D group: 460 ± 61.3 mg/dL; C group: 96.8 ± 10.2 mg/dL, $p < 0.001$). Kefir supplementation significantly reduced glucose levels in the DK group (DK group: 291.1 ± 44.2 mg/dL; compared to the D group, $p < 0.001$), whereas no significant difference was observed between the C and K groups ($p = 0.441$). Renal expression of TLR-2 and TLR-4 was markedly upregulated in diabetic rats ($p <0.001$), with TLR-2 and TLR-4 expression significantly attenuated following kefir treatment ($p <0.001$).*

Conclusion: *Kefir supplementation reduces blood glucose and renal TLR-2 and TLR-4 gene expression in diabetic rats, highlighting its therapeutic potential against diabetic nephropathy.*

Keywords: *Kefir, experimental diabetes mellitus, blood glucose, rat kidney, TLR-2 and TLR-4 gene expression.*

CORRESPONDING AUTHOR: Mustafa Kahraman Kosuyolu Cd. No:71, 34718 Kadikoy/Istanbul, Türkiye, mkahraman@istanbul.edu.tr

MK https://orcid.org/0000-0002-5775-5928 **EA** https://orcid.org/0000-0002-2924-811X **HN** https://orcid.org/0009-0004-5863-7247

EBB https://orcid.org/0000-0003-2759-3013 **ŞÖ** https://orcid.org/0000-0002-8809-7462

DELIVERING DATE: 04 / 12 / 2025 • **ACCEPTED DATE:** 16 / 02 / 2026

KEFİR TAKVİYESİNİN DİYABETİK SIÇANLARDA KAN GLUKOZU VE RENAL *TLR-2* VE *TLR-4* GEN EKSPRESYONU ÜZERİNDEKİ ETKİLERİ

ÖZET

Amaç: Bu çalışma, diyabet sıçan modelinde kefir takviyesinin kan glukozu düzenlemesi ve renal Toll-like reseptörler (*TLR-2 ve TLR-4*) ekspresyonu üzerindeki etkilerini araştırmayı amaçlamış olup, probiyotik-fermente gıdaların potansiyel anti-inflamatuar mekanizmalarına dair bulgular sunmaktadır.

Materyal ve Metot: Çalışmaya dahil edilen otuz beş sıçan rastgele dört gruba ayrıldı: kontrol (C), kefir verilen (K), diyabetik (D) ve kefir verilen diyabetik (DK). Hiperglisemiye bağlı komplikasyonları önlemek amacıyla, D ve DK grubu sıçanlara standart klinik kriterlere göre günde 1-3 ünite insülin aspart uygulanırken, K ve C gruplarına insülin verilmemiştir. Sıçanların kan glukoz seviyeleri ölçüldükten sonra sakrifiye edildi ve böbreklerin doku bütünlüğünün korunması için –80°C'de saklandı. Sıçan böbreklerinden RNA izole edildi ve cDNA sentezi için ters transkripsiyon yapıldı. *TLR-2* ve *TLR-4* ekspresyonunu değerlendirmek amacıyla kantitatif gerçek zamanlı PCR gerçekleştirildi.

Bulgular: Diyabetik sıçanlarda kan glukoz seviyeleri, kontrol grubuna kıyasla anlamlı şekilde artmıştır (D grubu: 460 ± 61,3 mg/dL; C grubu: 96,8 ± 10,2 mg/dL, $p<0,001$). Kefir takviyesi, DK grubunda glukoz seviyelerini anlamlı şekilde düşürmüştür (DK grubu: 291,1 ± 44,2 mg/dL; D grubuna kıyasla, $p<0,001$), oysa C ve K grupları arasında anlamlı bir fark gözlenmemiştir ($p=0,441$). Diyabetik sıçanlarda böbrek *TLR-2* ve *TLR-4* ekspresyonu anlamlı düzeyde artmıştır ($p<0,001$). Kefir tedavisi, her iki reseptörün ekspresyonunda da istatistiksel olarak anlamlı bir azalma sağlamıştır ($p<0,001$).

Sonuç: Kefir takviyesi, diyabetik sıçanlarda kan glikoz düzeyini ve renal *TLR-2* ile *TLR-4* gen ekspresyonunu azaltmakta olup, diyabetik nefropatiye karşı terapötik potansiyele işaret etmektedir.

Anahtar kelimeler: Kefir, deneysel diabetes mellitus, kan glukozu, sıçan böbreği, *TLR-2* ve *TLR-4* gen ekspresyonu.

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by elevated blood glucose levels. It occurs as a result of an absolute or partial deficiency of the hormone insulin.[1] The likelihood of a complication developing depends on the duration and severity of hyperglycemia.[2] Diabetes mellitus is a systemic disease that can lead to serious chronic complications such as diabetic nephropathy, retinopathy, neuropathy, and lower-extremity amputations. Diabetic nephropathy is characterized by persistent microalbuminuria, a marked decline in glomerular filtration rate, hypertension, and an increased risk of cardiovascular morbidity and mortality.[3] Elevated oxidative stress reduced glomerular filtration, and decreased sodium and water excretion have been reported to play key roles in its pathogenesis.[4] Numerous studies have demonstrated that inflammatory processes significantly contribute to the progression of diabetic nephropathy.[5,6] Approximately 20-50% of individuals with type 2 diabetes eventually develop diabetic nephropathy.[7]

Innate immunity constitutes the host's first and most rapid line of defense against invading microorganisms. Compared with adaptive immunity, it responds much more quickly, aiming to prevent the establishment of infection within the host and to promptly recognize and eliminate invading pathogens. Innate immunity has the capacity to distinguish host-derived components from microbial structures. One of the key protein families mediating this function is pattern recognition receptors (*PRRs*). Toll-like receptors (*TLRs*), a central member of the PRR family, play a pivotal role in pathogen recognition. Engagement of *TLRs* by pathogens triggers the activation of specific transcription factors, leading to the production and secretion of proinflammatory and antimicrobial cytokines and chemokines.[8] In diabetic rat models, the expression of cell adhesion molecules, chemokines, and proinflammatory cytokines in renal tissues has been observed to increase in association with albuminuria.[7] Recent studies have demonstrated that inflammation and Toll-like receptors, particularly *TLR-2* and *TLR-4*, play a central role in the development and progression of diabetic nephropathy. This mechanism involves increased chemokine production, infiltration of inflammatory cells into renal tissue, secretion of proinflammatory cytokines, and subsequent tissue damage.[9,10] Furthermore, downstream signaling mediated by *TLR-2* and *TLR-4* has been shown to induce insulin resistance in patients with type 2

diabetes through the upregulation of proinflammatory cytokine production.[9] Moreover, recent evidence shows that inhibition of *TLR-4* signaling (for example via wogonin) can alleviate renal inflammation and fibrosis in diabetic nephropathy by suppressing the JAK/STAT/AIM2 pathway.[11]

Complementary and alternative medicine (CAM) is increasingly popular among individuals with diabetes. Studies indicate that approximately 25% of diabetic patients use some form of complementary or alternative therapy during the course of their disease.[12] Although systematic reviews do not recommend the routine use of CAM in diabetes management, patient demand for alternative therapies remains significant.[13] The limitations of conventional medicine in addressing patient needs in conditions such as chronic pain and cancer may partly explain this interest.[14,15] While some studies have reported no significant benefit of probiotics on glycemic control in diabetes, others have observed notable reductions in HbA1c levels associated with kefir consumption.[16,17] Recent meta-analyses have shown that probiotic supplementation can improve glycemic control (HbA1c, fasting glucose, insulin, HOMA-IR) and positively affect lipid metabolism in patients with type 2 diabetes (e.g., WMD=–0.44% HbA1c).[18] Additionally, clinical evidence indicates that the consumption of probiotic-fermented milk has beneficial effects on metabolic parameters, including reductions in glucose and total cholesterol.[19]

Kefir is a lightly effervescent, whitish, sour sweet dairy product. It was developed through the natural fermentation of goat, sheep, and cow milk in the Caucasus region.[20,21] Compared to yogurt and other fermented dairy products, kefir is distinguished by its high content of probiotic microorganisms, B-complex vitamins, and digestive enzymes, which contribute to its unique functional properties.[22] Kefir and kefir-derived products have demonstrated antidiabetic effects in animal models of type 2 diabetes, including lowering blood glucose, enhancing insulin sensitivity, and preserving β-cell function.[23]

In our previous study, we demonstrated that kefir administration in diabetic rats led to significant reductions in blood glucose, creatinine, and urea levels. Additionally, kefir attenuated oxidative stress, reduced diabetes-induced renal damage, and slowed the progression of diabetic nephropathy.[4] In this study, we aimed to characterize changes in renal *TLR-2* and *TLR-4* gene expression during the progression of diabetic nephropathy in an experimental rat model and to assess the modulatory effects of kefir on these molecular and pathological processes.

MATERIAL and METHOD

This study was conducted at the Çanakkale Onsekiz Mart University Experimental Research Center with the approval of the Local Animal Experiments Ethics Committee (Approval number: 2013/02/06). Within the experimental protocol, a total of 40 male rats were randomly allocated into four groups: Control (C), Kefir-treated (K), Diabetes (D), and Kefir-treated Diabetic (DK), with n = 10 animals per group at baseline. All rats were provided with standard laboratory pellet diet and water ad libitum. Diabetes was induced in D and DK rats via streptozotocin injection, and animals with fasting blood glucose ≥ 250 mg/dl were included in the study. Kefir was administered by oral gavage to the K and DK groups at a standardized dose of 10 ml/kg/day for a total duration of 35 days, consistent with previously established protocols in experimental diabetic rat models, while C and D groups received saline for the same 35-day period; daily 1-3 units of insulin aspart (NovoRapid® 3 ml) were administered to D and DK rats solely to prevent severe hyperglycemia-related complications, including ketoacidosis, and animal mortality-not as a therapeutic intervention to normalize blood glucose- and both diabetic groups received insulin according to the same clinical criteria throughout the study period; rats were fasted overnight on day 35, and on day 36, blood samples were collected from the tail vein under standard conditions to measure fasting blood glucose levels; all animals were subsequently anesthetized and sacrificed for sample collection. In the original protocol, blood and urine samples were analyzed and published.[4] A portion of the kidney tissue was used in these analyses. The remaining kidney tissue was stored at –80 °C and, was re-evaluated within the scope of the original ethical approval for genetic analyses. During the re-evaluation of kidney tissues for genetic analyses, five samples that did not meet the predefined criteria for tissue integrity and RNA quality were excluded from the study. Of these excluded samples, two belonged to the Control group (C: n=2) and three to the Kefir-treated group (K: n=3). Consequently, renal genetic analyses were performed on a total of 35 rats. Frozen rat kidneys that met the required quality and tissue-integrity criteria were included in the analysis. The glucose values reported here correspond specifically to this subset of rats. The genetic analyses of this study were performed at the molecular laboratory of the Medical Genetics Division, Department of Internal Medicine, Istanbul University Faculty of Medicine.

In this study, Turkish kefir (Radoha Kefir® Secen Natural Food Co., Ltd., Bursa, Turkey) was used. The kefir was prepared using 5% kefir grains, and the dominant bacterial genus was Lactobacillus. The most prevalent species included Lactobacillus kefiranofaciens, Lactobacillus buchneri, Lactobacillus helveticus, Lactobacillus casei, and Streptococcus thermophilus. According to the manufacturer, following 24 hours of fermentation, the Lactobacillus population reached 10^8 CFU/ml. The product was stored at 5±1 °C and used 7-14 days after production. Previous studies have reported that Lactobacillus constitutes more than 90% of the microbial population in Turkish kefir.[24].

RNA Isolation

Kidney tissue (400 mg) was placed into a homogenization tube containing 1 ml of 0.1 M Tris-HCl (pH 8.0) treated with diethyl pyrocarbonate (DEPC) and 300 mg of 1 mm glass beads and homogenized at 7,000 rpm for 2 minutes. RNA was extracted using the acid guanidinium thiocyanate-phenol-chloroform method as described by Chomczynski and Sacchi.[25] Briefly, 500 µl of the homogenate was transferred to a new tube and mixed with 500 µl of denaturation solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7; 0.5% sarcosyl; 0.1 M 2-mercaptoethanol) and incubated at room temperature for 5 minutes. Subsequently, 150 µl of chloroform was added, the mixture was manually shaken for 15 seconds and incubated for 3 minutes at room temperature. Samples were centrifuged at 12,000xg for 15 minutes at 4°C, and the upper aqueous phase was transferred to a new tube. RNA was precipitated by adding 400 µl of 2-propanol and incubating at room temperature for 10 minutes. Following centrifugation at 12,000xg for 10 minutes at 4°C, the supernatant was discarded, and the pellet was washed with 1 ml of 75% ethanol. After mixing, samples were centrifuged at 7,500xg for 5 minutes at 4°C, the ethanol was removed, and residual ethanol was evaporated for 10 minutes. The RNA pellet was dissolved in 50 µl of RNase-free deionized water and stored at –80°C until further use.

Reverse Transcription

12 microliters of RNA (20 ng/µl) were mixed with 4 µl of oligo(dT) primers and incubated at 70°C for 10 minutes, followed by 5 minutes on ice. Subsequently, 8 µl of 5x reaction buffer (250 mM Tris-HCl, pH 8.3; 375 mM KCl; 15 mM $MgCl_2$; 50 mM DTT), 2 µl of dNTP mix (40 mM), 2 µl of reverse transcriptase (200 U/µl), and 28 µl of sterile deionized water were added. The reaction mixture was incubated at 37°C for 60 minutes, followed by 10 minutes at 60°C. The resulting cDNA was stored at –20°C until further use.

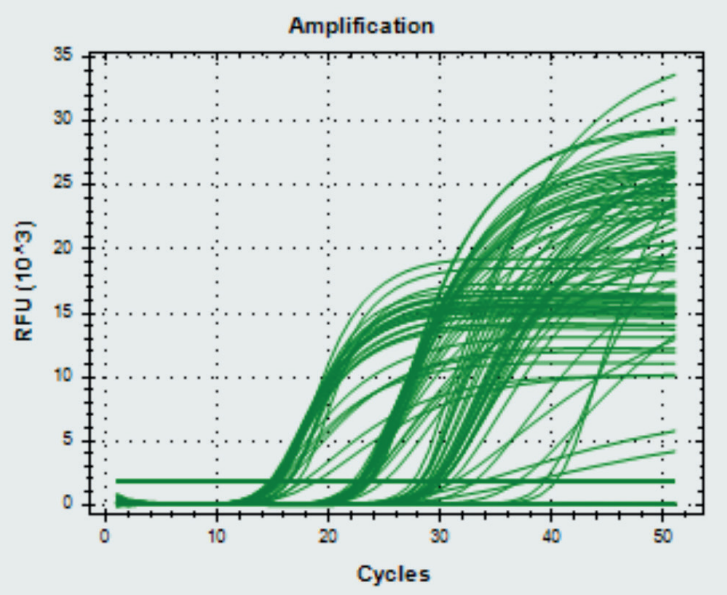

Figure 1. qPCR amplification curves

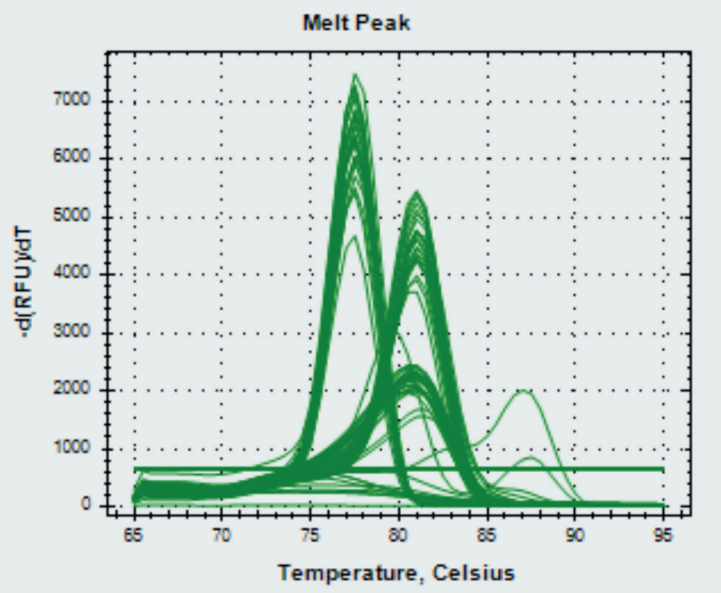

Figure 2. qPCR melting curves

Quantitative Real-Time PCR (qPCR) Protocol

The The relative expression levels of Toll-Like Receptor 2 (TLR-2) and Toll-Like Receptor 4 (TLR-4) genes in rat samples, normalized to the housekeeping gene G3PDH, were quantified using real-time polymerase chain reaction (qPCR) as described by Schultz et al. (2007).[26] The qPCR primers used for this analysis are listed in Table 1. Reactions were performed on a Bio-Rad CFX Connect system and contained 1x.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1x reaction buffer,

Table 1. QPCR Primers

Named	5'-3' DNA Sequence
G3PDH-77F	AGAGAGAGGCCCTCAGTTGCT
G3PDH-77R	TGGAATTGTGAGGGAGATGCT
TLR2-75F	GTACGCAGTGAGTGGTGCAAGT
TLR2-75R	GGCCGCGTCATTGTTCTC
TLR4-107F	AATCCCTGCATAGAGGTACTTCCTAAT
TLR4-107R	CTCAGATCTAGGTTCTTGGTTGAATAAG

Table 2. Mean blood glucose levels of rats in the two control and two experimental groups are presented.

Group	*n*	Blood Glucose (mg/dL, mean ± SEM)	Statistical Comparison
Control (C)	8	96.8 ± 10.2	Reference
Kefir-treated Control (K)	7	157 ± 7.1	K vs. C: $p = 0.441$
Diabetes (D)	10	460 ± 61.3	D vs. C: $p < 0.001$
Kefir-treated Diabetic (DK)	10	291.1 ± 44.2	DK vs. D: $p < 0.001$

C: Control group, **K:** kefir-treated Control group, **D:** Diabetic group, **DK:** Kefir-treated Diabetic group. Values are mean ± SEM. Diabetes increased glucose (D vs. C, $p < 0.001$); kefir reduced it in diabetic rats (DK vs. D, $p < 0.001$) but not in controls (K vs. C, $p = 0.441$).

0.1 U proofreading Hot-Start Taq DNA polymerase, 1x EvaGreen, 5 ng/µl template cDNA, and 0.5 µM of each primer. Amplification was carried out with an initial denaturation at 95 °C for 10 minutes, followed by 50 cycles of 95°C for 10 seconds and 57°C for 30 seconds (Figure 1). To confirm the specificity of the amplified products, a melting curve analysis was performed between 55°C and 95°C (Figure 2).

For all template cDNAs, the RNA templates from which the cDNAs were synthesized were included as negative controls in the qPCR reactions to confirm that amplification originated from cDNA rather than genomic DNA contamination. To assess reaction efficiency, one of the template DNAs was serially diluted, and the efficiency of the reaction was calculated. The relative expression levels of the target genes normalized to the G3PDH gene were determined using the 2^−ΔΔCt method.[27]

Statistics

Data were analyzed using GraphPad Prism 5.0 (La Jolla, California, USA). The normality of numerical variables was assessed using the Shapiro-Wilk test. Blood glucose data were found to be normally distributed and were therefore analyzed using parametric tests. For normally distributed data, comparisons between groups were performed using independent sample t-tests or one-way ANOVA. Homogeneity of variances was evaluated using Levene's test or Bartlett's test. When variances were unequal, non-parametric tests or Welch's correction were applied. For multiple group comparisons with significant differences, post-hoc analyses were conducted using Tukey's test, and pairwise p-values are reported explicitly for each group comparison. Categorical variables were presented as frequencies and percentages, and numerical data were expressed as mean ± standard error of the mean (SEM) throughout the manuscript. A significance level of $p<0.05$ was considered statistically significant.

Specifically, for *TLR-2* and *TLR-2* gene expression data, variance homogeneity tests were performed, and appropriate corrections were applied when necessary. Effect sizes were calculated using eta squared (η^2) rather than r^2, which is more appropriate for ANOVA. The validity of qPCR results was confirmed through negative control samples showing no amplification and through melting curve analyses.

RESULTS

The measured mean blood glucose levels for all groups are presented in Table 2. A statistically significant difference was observed among the four groups ($p<0.001$). Pairwise comparisons using Tukey's post-hoc test revealed significant differences between the D and C groups, D and K groups, and DK and D groups ($p<0.001$ for each), while no significant difference was detected between the C and K groups ($p=0.441$). Blood glucose levels differed significantly among the experimental groups. Diabetic rats exhibited markedly higher glucose levels compared to controls, whereas administration of kefir to diabetic rats significantly improved glucose levels relative to untreated diabetic animals. In non-diabetic rats, kefir supplementation did not produce a statistically significant change in mean glucose levels.

Analysis of TLR gene expression revealed significant differences among groups. *TLR-2* expression varied significantly ($F(3,36)=23.21$, $p<0.001$, $\eta^2=0.659$), with unequal variances detected across groups (Bartlett's test, $p<0.001$). *TLR-4* expression also differed significantly among groups ($F(3.36)=60.48$, $p<0.001$, $\eta^2=0.834$), with no evidence of unequal variances (Bartlett's test, $p=0.072$). In all qPCR reactions, negative controls and RNA-only templates showed no target amplification, confirming the absence of genomic DNA contamination. Melting curve analyses indicated that only the expected products were amplified, and all reactions targeting the G3PDH housekeeping gene yielded the anticipated Tm values, confirming successful RNA isolation, cDNA synthesis, and amplification. The expression levels of the *TLR-2*

and *TLR-4* genes are presented in Figures 3 and 4, respectively.

No amplification was observed in reactions where only RNA was used as the template, indicating that genomic DNA was effectively removed during RNA isolation. Similarly, no amplification occurred in the no-template negative controls; however, a qPCR product was detected at 87°C in these controls. Since this melting temperature did not correspond to the expected target product range (77.5-81.5°C), it was concluded that no target amplification occurred in the negative controls. All qPCR reactions targeting the G3PDH gene yielded the expected melting temperatures, confirming successful amplification. These results demonstrate that RNA isolation, cDNA synthesis, and qPCR procedures were effectively performed.

DISCUSSION

This study investigated the effects of kefir supplementation on glycemic regulation, inflammation, and Toll-like receptor (TLR) expression in the renal tissue of diabetic rats, thereby exploring the potential role of probiotic-fermented products in the progression of diabetic nephropathy (DN). Our findings demonstrate that kefir significantly reduced blood glucose levels in diabetic rats and attenuated the diabetes-induced upregulation of renal *TLR-2* and *TLR-4* expression. These results align with the established literature indicating that diabetes activates inflammatory pathways in the kidney and that TLR-mediated signaling plays a pivotal role in this process.

The central involvement of *TLR-4* in the pathophysiology of diabetic nephropathy is strongly supported by both human and experimental evidence. Increased renal tubular *TLR-4* expression has been documented in patients with DN, correlating with interstitial macrophage infiltration and declining glomerular filtration rate (GFR).[28] In streptozotocin-induced diabetic mice, genetic deletion of *TLR-4* mitigated albuminuria, inflammation, and fibrosis, indicating a causative role for *TLR-4* mediated signaling in renal injury.[29] Additionally, albumin has been shown to induce a pro-inflammatory response in proximal tubular cells via the HSP70–*TLR-4* axis; blockade of *TLR-4* reduced inflammation and tubular injury.[30] Preclinical and review data further highlight that endogenous danger signals such as HMGB1 activate *TLR-2* and *TLR-4* in diabetic kidneys, perpetuating inflammatory and fibrogenic cascades.[31] The pronounced increase in renal *TLR-2* and *TLR-4* gene expression observed in our diabetic group is therefore consistent with these mechanisms and further confirms the inflammatory activation characteristic of DN.

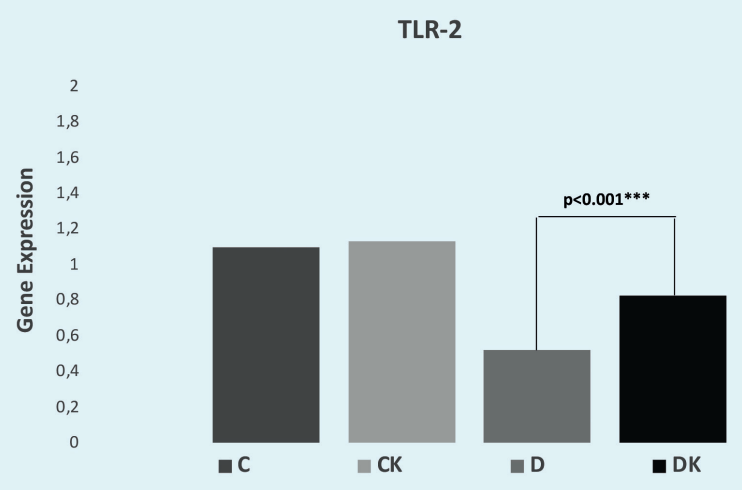

Figure 3. The expression levels of the TLR-2 gene

C: Control group, **CK:** Kefir-treated Control group, **D:** Diabetic group, **DK:** Kefir-treated Diabetic group

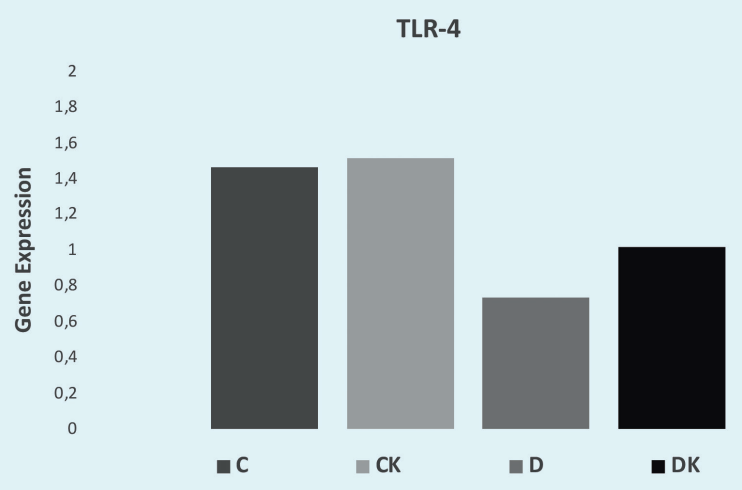

Figure 4. The expression levels of TLR-4

C: Control group, **CK:** Kefir-treated Control group, **D:** Diabetic group, **DK:** Kefir-treated Diabetic group

The modulatory effects of kefir on Toll-like receptor signaling can be attributed to its complex microbial ecosystem. Metagenomic studies of kefir grains have revealed that Lactobacillus kefiranofaciens and Lactobacillus helveticus are among the dominant species, accompanied by Lactococcus, Leuconostoc, acetic acid bacteria, and yeasts, all embedded within a polysaccharide matrix.[32] These bacteria produce bioactive compounds such as short-chain fatty acids and exopolysaccharides, which are known to interact with mucosal immunity and influence Toll-like receptor pathways.[33] Through gut–kidney crosstalk, these metabolites could suppress proinflammatory TLR activation, potentially accounting for the reduced expression of *TLR-2* and *TLR-4* observed in our diabetic nephropathy model.

The beneficial effects of probiotics on metabolic diseases have been increasingly recognized. A high-quality systematic review by Liu Y et al. demonstrated that probiotic and fermented-food consumption significantly improves glycemic control, reduces fasting glucose and HbA1c levels, improves insulin sensitivity, and modulates systemic inflammation.[11] These outcomes are consistent with the marked decrease in blood glucose levels observed in the kefir-treated diabetic rats.

Substantial evidence indicates that kefir confers both metabolic benefit and may exert potential renoprotective effects. Bioactive peptides produced during kefir fermentation have been shown to exert strong antioxidative and anti-inflammatory effects, which can modulate immune responses and reduce cellular stress in various experimental models. For instance, Chen et al. reported that kefir-derived peptides significantly attenuated renal oxidative stress and inflammation in hypertensive rats, leading to improved kidney function.[34] Similarly, Aires et al., demonstrated that kefir peptides reduced systemic markers of inflammation and reactive oxygen species, highlighting their potential to influence TLR-mediated signaling pathways.[35] These bioactive components, combined with kefir's complex microbial profile, may contribute to the observed downregulation of renal *TLR-2* and *TLR-4* expression in diabetic rats, although this alone does not constitute direct evidence of renoprotection. These findings provide mechanistic insight into how kefir supplementation could influence diabetes-associated renal inflammation by modulating oxidative stress, inflammation, and innate immune activation, yet further studies evaluating renal histopathology and functional biomarkers are needed to confirm true renoprotective effects.

The gut microbiota has emerged as a crucial regulator of renal inflammation. Dysbiosis in chronic kidney disease can drive oxidative stress, leaky gut, and systemic inflammation.[36] Given that diabetic nephropathy involves similar inflammatory mechanisms, it is plausible that kefir mediates potential protective effects on the kidney via gut-derived metabolites that suppress TLR–mediated signaling. Recent evidence indicates that gut dysregulation contributes to renal damage through microbial-metabolite–mitochondrial crosstalk in diabetic kidney disease.[37] Moreover, reviews have highlighted the dual role of gut microbiota in renal fibrosis, showing that imbalances can exacerbate inflammation and oxidative stress, while restoration of beneficial taxa and short-chain fatty acids may attenuate these processes.[38] Our data, which demonstrate reduced expression of *TLR-2* and *TLR-4* following kefir supplementation, are consistent with this emerging paradigm, supporting a cautious interpretation that kefir may modulate inflammation-driven renal injury rather than definitively prevent it.

Limitations

This study has several limitations that should be acknowledged. The relatively small sample size may restrict the statistical power and generalizability of the findings. Although significant changes in renal *TLR-2* and *TLR-4* mRNA expression were observed, the lack of protein-level validation and downstream pathway analyses, such as NF-κB activation, cytokine profiling, or fibrosis markers, limits the interpretation of the functional significance of these molecular changes. Additionally, kefir is a biologically variable fermented product, and differences in microbial composition between batches or commercial sources may affect reproducibility. Importantly, supportive insulin therapy was administered only to the D and DK groups according to uniform clinical criteria. Although glucose levels in the DK group were slightly lower than in the D group, this standardized application is unlikely to have systematically confounded the assessment of kefir's effects. Finally, the absence of gut microbiota analysis prevents determination of whether the observed renal effects were mediated directly in kidney tissue or indirectly through gut-derived metabolites and host–microbiome interactions.

CONCLUSION

This study demonstrates that kefir supplementation has significant beneficial effects on glycemic regulation and renal inflammation in diabetic rats. The observed substantial reduction in blood glucose levels, coupled with decreased expression of renal *TLR-2* and *TLR-4*, indicates that kefir can modulate both metabolic and inflammatory pathways implicated in the progression of diabetic nephropathy. These findings highlight the therapeutic potential of kefir as a natural, probiotic-based intervention to alleviate diabetes-induced renal injury and systemic metabolic dysregulation.

Furthermore, the results suggest that bioactive components within kefir may contribute to the modulation of inflammatory signaling and support metabolic homeostasis. Although the underlying mechanisms require further clarification, these data provide a foundation for future research exploring kefir's potential to enhance glycemic control and renal function in diabetic conditions.

*The authors declare that there are no conflicts of interest.

REFERENCES

1. TEMD Diyabet Çalışma Grubu. Diabetes Mellitus ve Komplikasyonlarının Tanı, Tedavi ve İzlem Kılavuzu-2019. [cited 2025 Nov 2]. Available from: http://temd.org.tr
2. Hsueh WA, Moore L, Bryer-Ash M. Contemporary diagnosis and management of type 2 diabetes. Handbooks Health Care Co; 2004.
3. Satman I, Yilmaz T, Sengül A, et al. Population-based study of diabetes and risk characteristics in Turkey: results of the Turkish Diabetes Epidemiology Study (TURDEP). Diabetes Care 2002; 25: 1551-1556.
4. Kahraman M, Ertekin YH, Satman İ. The effects of kefir on kidney tissues and functions in diabetic rats. Probiotics Antimicrob Prot 2021; 13: 375-382.
5. Duran-Salgado MB, Rubio-Guerra AF. Diabetic nephropathy and inflammation. World J Diabetes 2014; 5: 393-398.
6. Xiao X, Ma B, Dong B, et al. Cellular and humoral immune responses in the early stages of diabetic nephropathy in NOD mice. J Autoimmun 2009; 32: 85-93.
7. Hoogeveen EK. The epidemiology of diabetic kidney disease. Kidney Dial 2022; 2: 433-442.
8. Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al. Autoimmunity: from bench to bedside. Bogota (Colombia): El Rosario University Press; 2013 Jul 18.
9. Aly RH, Ahmed AE, Hozayen WG, et al. Patterns of toll-like receptor expressions and inflammatory cytokine levels and their implications in the progress of insulin resistance and diabetic nephropathy in type 2 diabetic patients. Front Physiol 2020; 11.
10. Mudaliar H, Pollock C, Panchapakesan U. Role of toll-like receptors in diabetic nephropathy. Clin Sci 2014; 126: 685-694.
11. Liu Y, Zhang M, Zeng L, et al. Wogonin upregulates SOCS3 to alleviate the injury in diabetic nephropathy by inhibiting TLR4-mediated JAK/STAT/AIM2 signaling pathway. Mol Med 2024; 30: 78.
12. Hunt KJ, Coelho HF, Wider B, et al. Complementary and alternative medicine use in England: results from a national survey. Int J Clin Pract 2010; 64: 1496-1502.
13. Posadzki P, Lee MS, Ernst E. Complementary and alternative medicine for diabetes mellitus: an overview of systematic reviews. Focus Altern Complement Ther 2012; 17: 142-148.
14. Mahmoudian SA, Ebrahim-Babaei M, Jafari M. The reasons for and satisfaction from using acupuncture in Isfahan. J Isfahan Med Sch 2012; 29: 2280-2288.
15. Chiu L, Balneaves L, Barroetavena MC, et al. Use of complementary and alternative medicine by Chinese individuals living with cancer in British Columbia. J Complement Integr Med 2005; 2: 1-16.
16. Barengolts E, Smith ED, Reutrakul S, et al. The effect of probiotic yogurt on glycemic control in type 2 diabetes or obesity: a meta-analysis of nine randomized controlled trials. Nutrients 2019; 11: 671.
17. Ostadrahimi A, Taghizadeh A, Mobasseri M, et al. Effect of probiotic fermented milk (kefir) on glycemic control and lipid profile in type 2 diabetic patients: a randomized double-blind placebo controlled clinical trial. Iran J Public Health 2015; 44: 228-237.
18. Hejazi N, Ghalandari H, Rahmanian R, et al. Effects of probiotics supplementation on glycemic profile in adults with type 2 diabetes mellitus: a grade-assessed systematic review and dose-response meta-analysis of randomized controlled trials. Clin Nutr ESPEN 2024; 64: 133-143.
19. Zikou E, Dovrolis N, Dimosthenopoulos C, et al. The effect of probiotic supplements on metabolic parameters of people with type 2 diabetes in Greece-a randomized, double-blind, placebo-controlled study. Nutrients 2023; 15: 4663.
20. Yilmaz B, Sharma H, Melekoglu E, Ozogul F. Recent developments in dairy kefir-derived lactic acid bacteria and their health benefits. Food Biosci 2022; 46: 101592.
21. Guzel-Seydim ZB, Kok-Tas T, Greene AK, Seydim AC. Review: functional properties of kefir. Crit Rev Food Sci Nutr 2011; 51: 261-268.
22. Leite AMDO, Miguel MAL, Peixoto RS, et al. Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. Braz J Microbiol 2013; 44: 341-349.
23. Talib N, Mohamad NE, Yeap SK, et al. Anti-diabetic effect of Lactobacillus paracasei isolated from Malaysian water kefir grains. Probiotics Antimicrob Proteins. 2024; 16: 2161-2180.
24. Nalbantoglu U, Cakar A, Dogan H, et al. Metagenomic analysis of the microbial community in kefir grains. Food Microbiol 2014; 41: 42-51.
25. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-159.
26. Le Mandat Schultz A, Bonnard A, Barreau F, et al. Expression of TLR-2, TLR-4, NOD2 and pNF-κB in a neonatal rat model of necrotizing enterocolitis. PLoS One 2007; 2: 1102.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCT method. Methods 2001; 25: 402-408.
28. Lin M, Yiu WH, Wu HJ, et al. Toll-like receptor 4 promotes tubular inflammation in diabetic nephropathy. J Am Soc Nephrol 2012; 23: 86-102.
29. Ma J, Chadban SJ, Zhao CY, et al. TLR4 activation promotes podocyte injury and interstitial fibrosis in diabetic nephropathy. PLoS One 2014; 9: 97985.
30. Jheng HF, Tsai PJ, Chuang YL, et al. Albumin stimulates renal tubular inflammation through an HSP70-TLR4 axis in mice with early diabetic nephropathy. Dis Model Mech. 2015; 8: 1311-1321.
31. Lin M, Tang SC. Toll-like receptors: sensing and reacting to diabetic injury in the kidney. Nephrol Dial Transplant 2014; 29: 746-754.
32. Zeng X, Wang Y, Jia H, et al. Metagenomic analysis of microflora structure and functional capacity in probiotic Tibetan kefir grains. Food Res Int 2022; 151: 110849.
33. Ding F, Stoyanova LG, Netrusov AI. Microbiome and metabiotic properties of kefir grains and kefirs based on them. Microbiology 2022; 91: 339-355.
34. Chen YH, Chen HL, Fan HC, et al. Anti-inflammatory, antioxidant, and antifibrotic effects of kefir peptides on salt-induced renal vascular damage and dysfunction in aged stroke-prone spontaneously hypertensive rats. Antioxidants 2020; 9: 790.
35. Aires R, Gobbi Amorim F, Côco LZ, et al. Use of kefir peptide (Kef-1) as an emerging approach for the treatment of oxidative stress and inflammation in 2K1C mice. Food Funct 2022; 13: 1965-1974.

36. Lau WL, Chang Y, Vaziri ND. The consequences of altered microbiota in immune-related chronic kidney disease. Nephrol Dial Transplant 2021; 36:1791-1798.

37. Ma L, Zhang L, Li J, et al. The potential mechanism of gut microbiota–microbial metabolites–mitochondrial axis in progression of diabetic kidney disease. Mol Med 2023; 29: 148.

38. Cao C, Zhu H, Yao Y, Zeng R. Gut dysbiosis and kidney diseases. Front Med 2022; 9: 829349.