Research Article

# High-intensity interval training and royal jelly synergistically attenuate insulin resistance and renal inflammatory markers (TNF-α and NF-κB) in experimental diabetes

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## Abstract

Exercise and the use of anti-inflammatory supplements are effective in controlling inflammation and tissue damage in diabetes. Therefore, the aim of the present study was to investigate the effect of 8 weeks of interval training and royal jelly on the expression of renal inflammatory genes and insulin resistance in diabetic rats. Thirty-two male Wistar rats were used in this study. Diabetes was induced by a high-fat diet for 20 weeks followed by intraperitoneal injection of freshly prepared STZ solution in saline (25 mg/kg). Then the rats were randomly divided into 4 groups: Diabetes (DI), DI+ HIIT, DI+ royal jelly (RJ), and DI+ HIIT+ RJ. Royal jelly groups were given royal jelly at a dose of 100 mg/kg diluted in distilled water and gavage 5 days a week. The exercise program consisted of eight weeks of HIIT training, five sessions per week with a gradual increase in intensity intervals from 22 to 38 meters per minute (80 to 90% of Vo2max) and rest intervals at a speed of 16 to 22 meters per minute (50 to 56% of Vo2max). At the end of the research, rats were euthanized and kidney tissue was removed to measure the expression of TNF-α and NF-κB genes. The DI+HIIT (p=0.0006), DI+RJ (p=0.0011) and DI+HIIT+RJ (p<0.0001) groups showed a significant decrease in HOMA-IR compared to the diabetes control group. The DI+HIIT, DI+RJ and DI+HIIT+RJ groups showed a significant decrease in TNF-α and NF-κB gene expression in kidney tissue compared to the diabetes control group. In conclusion, 8 weeks of HIIT and/or royal jelly supplementation significantly ameliorated insulin resistance and suppressed renal pro-inflammatory gene expression (TNF-α, NF-κB) in diabetic rats, with the combined approach showing the greatest efficacy.

*Key Words:* Kidney, Inflammation, Insulin resistance, Diabetes

## Introduction

Chronic high blood sugar in diabetes progressively damages the kidneys by impairing their delicate vasculature and nephrons (Kumar et al., 2023). This pathological process, termed diabetic nephropathy, is characterized by increased pressure within the filtering units (glomeruli), leading to protein leakage into the urine (albuminuria) and declining renal function (Amanat et al., 2025). Over time, structural and functional changes within the glomerulus include thickening of the basement membrane, expansion of mesangial cells, and buildup of matrix deposits like Kimmelstiel-Wilson nodules, which eventually disrupt filtration and drive the progression to chronic kidney disease and, in many cases, end-stage renal disease. A major contributor to this destructive cycle is renal inflammation. Key mediators such as tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa B (NF-kB) become activated in response to cell stress and hyperglycemia (Singh et al., 2019). TNF-α acts as a potent proinflammatory cytokine, amplifying the production of other inflammatory mediators and promoting fibrosis, glomerular injury, and apoptosis of kidney cells. NF-kB, a transcription factor, is triggered by high glucose and TNF-α signaling in renal cells, perpetuating the expression of genes involved in inflammation and immune responses. Studies reveal that inhibition of TNF-α can significantly reduce kidney fibrosis, albuminuria, and renal inflammation, highlighting its pathogenic role in diabetic kidney injury (Taguchi et al., 2021).

Insulin resistance, a hallmark of type 2 diabetes and frequently present in kidney disease, worsens renal outcomes through several mechanisms (Lee et al., 2022). Persistently high insulin levels (hyperinsulinemia) provoke sodium retention, vascular remodeling, proliferation of mesangial cells, and stimulation of profibrotic pathways such as transforming growth factor-beta (TGF-β) (Zhang et al., 2021). Insulin resistance also accelerates renal fibrosis and proteinuria, independent of overt diabetes, with multiple clinical studies documenting faster progression of chronic kidney disease (CKD) and declining

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**Received 22 March 2025; Accepted 4 June 2025**

glomerular filtration rates (GFR) in patients with reduced insulin sensitivity (Adeva-Andany et al., 2023). The constellation of inflammation, oxidative stress, and metabolic disturbances arising from insulin resistance all act synergistically to accelerate diabetic renal damage (Spoto et al., 2016).

Exercise training is increasingly recognized as a powerful non-pharmacological strategy to mitigate diabetes-induced kidney damage and modulate renal inflammation (Bishop et al., 2023). Scientific studies show that moderate aerobic exercise improves kidney function, reduces microalbuminuria, restores oxidative balance, and increases nitric oxide bioavailability in both animal models and patients with diabetes (Aldahr & Abd El-Kader, 2022). By engaging key metabolic pathways such as AMPK and IRS1/PI3-K/AKT/GLUT4, exercise enhances insulin sensitivity and glycemic control (Fan et al., 2024), which helps slow the progression of diabetic nephropathy. One crucial effect of regular exercise is its anti-inflammatory action: moderate-intensity aerobic training suppresses renal inflammatory markers, notably tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa B (NF-kB) (Malheiro et al., 2024). Studies have demonstrated that aerobic training decreases NF-kB gene expression and its downstream pro-inflammatory cytokines in the kidneys, reduces infiltration of macrophages and lymphocytes, and attenuates fibrotic signaling such as TGF-β1. Furthermore, exercise upregulates antioxidant defense (via Nrf2) and activates SIRT1, which further inhibits NF-kB acetylation, constraining the inflammatory feedback loop. These changes collectively contribute to improved renal structure and function, reduced albuminuria, and decreased risk of progression to end-stage renal disease in diabetes. The evidence also suggests exercise should be individually tailored to maximize benefits and avoid possible risks in those with advanced renal impairment. Exercise's direct reduction of renal oxidative stress and inflammation, primarily via the NF-kB and TNF-α pathways, underscores its central role in protecting the diabetic kidney and highlights the importance of integrating physical activity into comprehensive diabetes care (Amaral et al., 2020).

Recent scientific studies indicate that royal jelly (RJ), the unique secretion from honey bees, exerts protective effects on kidneys damaged by diabetes, primarily through its potent antioxidant and anti-inflammatory actions. Experimental research in diabetic animal models demonstrated that oral supplementation of RJ significantly improved kidney histology and biochemical parameters, including reductions in urine protein and urea, alongside restoration of antioxidant enzyme activity in renal tissue. These effects were attributed to RJ's ability to scavenge excess free radicals generated in diabetes, which are key mediators of oxidative stress and contribute to the progression of diabetic nephropathy (Ghanbari et al., 2015). In clinical research, royal jelly supplementation has also shown promise in improving insulin sensitivity and lowering markers of oxidative stress in patients with type 2 diabetes. Over several weeks of administration, RJ reduced the insulin resistance index (HOMA-IR), improved glycemic status, and boosted total antioxidant capacity in the serum of diabetic individuals. Mechanistically, royal jelly and its bioactive compounds—such as hesperetin, naringenin, genistein, and 10-hydroxy-2-decenoic acid—act on various metabolic pathways to enhance insulin action, suppress pro-inflammatory cytokines, and upregulate antioxidant defenses (El-Seedi et al., 2024).

Based on research it seems that the anti-inflammatory effect of exercise and RJ can improve kidney tissue and reduce kidney inflammation. Therefore, the aim of the present study was to investigate the effect of 8 weeks of interval training and royal jelly on the expression of renal inflammatory genes and insulin resistance in diabetic rats.

## Materials and methods

### Animals

The statistical population of the present study consisted of 4-6 week old male Wistar rats. After obtaining the rats from Royan Institute (Iran), they were housed in the animal house of Razi Laboratory of Azad University of Science and Research (Tehran, Iran). The average weight of the rats at the beginning of the study was 170±30. After two weeks of adaptation to the environment and reaching a weight of 193±20 grams, they were placed on a high-fat diet. All rats were kept in the laboratory under controlled light conditions (12 hours of light and 12 hours of darkness) with a temperature of (22±3°C) and a humidity range of 30 to 60%, and then were fed a high-fat diet (45 to 60% fat) purchased from Royan Biotechnology Research Institute (Tehran, Iran) for 5 months. This study was approved by the Medical Ethics Committee of Azad University of Science and Research under the ethics code IR.IAU.SRB.REC.1402.292.

### Diabetes induction (High-fat diet and STZ)

Type 2 diabetes was induced in male Wistar rats using a combination of a high-fat diet (HFD) and a low-dose streptozotocin (STZ) injection, as previously described (Gheibi et al., 2016; Srinivasan et al., 2005). After a one-week acclimatization period, all rats were fed a HFD for a total of 20 weeks. The diet, prepared by the Royan Biotechnology Research Institute, provided 45% of total energy from fat (animal oil) for the first 12 weeks (composition: 24% fat, 24% protein, 41% carbohydrate per 100 g), followed by a diet providing 60% of energy from fat for the remaining 8 weeks.

After the 20-week HFD feeding period, rats received a single intraperitoneal injection of freshly prepared STZ (25 mg/kg in saline solution). One week post-injection, fasting blood glucose

(FBG) was measured from a small tail vein nick using a glucometer. Rats with FBG levels between 150 and 400 mg/dL were considered diabetic and included in the study.

To further confirm the diabetic phenotype, a subset of 10 rats was randomly selected for additional analysis; plasma insulin levels were measured, and the insulin resistance index (HOMA-IR) was calculated, confirming a state of insulin resistance and impaired glucose tolerance (Gheibi et al., 2016; Srinivasan et al., 2005).

A total of 32 confirmed diabetic rats were then randomly divided into four experimental groups (n=8 per group): diabetic control (DC), diabetes + high-intensity interval training (HIIT), diabetes + royal jelly (RJ), and diabetes + HIIT + RJ.

All necessary measures were taken to minimize animal suffering, including providing timely food and water, regular cage cleaning, maintaining an appropriate density of 3-4 rats per cage, and ensuring a quiet and suitable environment.

## Royal jelly

During the research period, rats in the royal jelly (RJ) and high-intensity interval training + royal jelly (HIIT+RJ) groups received royal jelly via gavage at a dose of 100 mg/kg, diluted in distilled water, five days per week prior to training sessions. To account for the stress of the gavage procedure, rats in the control and HIIT-only groups received an equivalent volume of distilled water (placebo) on the same schedule.

Royal jelly, which contains abundant phenolic compounds from the flavonoid family (e.g., quercetin, kaempferol, apigenin, and luteolin), was analyzed and stored at -20°C. It was dissolved in distilled water according to the required dose immediately prior to administration, following established protocols (Asgari et al., 2017; Waykar Bhalchandra & Alqadhi, 2018).

## HIIT

The program consisted of eight weeks of HIIT training, five sessions per week with a gradual increase in intensity intervals from 22 to 38 meters per minute (80 to 90% of $VO_{2max}$) and rest intervals at a speed of 16 to 22 meters per minute (50 to 56% of $VO_{2max}$) and a time of 15 to 34 minutes, in the form of treadmill running; so that the running time increased from 16 minutes in the first week to 34 minutes in the eighth week. One week before the start of the protocol, the rats walked on the treadmill three days a week at a speed of five meters per minute with a zero percent incline for 10, 12, and 15 minutes. The control group also walked on the treadmill in the same manner during the protocol. The maximum speed was determined using the protocol of Rodriguez et al. (2007). To measure maximal oxygen consumption ($VO_{2max}$), due to the lack of access to direct instruments (such as a respiratory gas analyzer) and based on previous research, an indirect protocol was used with great accuracy. In this way, every two weeks, after a five-minute warm-up, the rat started running at a speed of 10 meters per minute, then at a speed of 15 meters per minute for two minutes, and the speed was increased by three meters per minute every three minutes until each of the rat that could not continue and remained on the shocker and reached exhaustion, that speed was considered as their maximum speed, and the maximum speed was considered for an exercise intensity between 80 and 95% of MERT (Rodrigues et al., 2007).

## Laboratory measurements

After 48 hours from the last training session and after 12 hours of fasting, after the rat were anesthetized with ether under pain-free conditions and without painful and chronic disorders in the animal, they were anesthetized and sacrificed, and blood was collected from their hearts and then tissue was taken. Blood samples were stored at -20 degrees Celsius. Glucose was measured using an auto-analyzer and insulin was measured using a special kit from Pars Azmon Company. Insulin resistance index (HOMA-IR) was calculated using the following formula:

Fasting glucose (mg/dL) × fasting insulin (μIU/mL)/405 = Insulin resistance (HOMA-IR)

## Gene expression analysis in kidney tissue (TNF-α and NF-κB)

For molecular analysis of TNF-α and NF-κB gene expression, RNA was extracted from kidney tissue of all studied groups using a commercial kit (Qiagen, Germany) according to the manufacturer's protocol. Briefly, approximately 30 mg of tissue was homogenized in 200 μL of TRIzol reagent. The homogenate was incubated at -80°C for 24 hours. Subsequently, the samples were thawed and 100 μL of chloroform was added, followed by vigorous vortexing for 1 minute. The resulting solution was centrifuged at 12,000 × g for 15 minutes at 4°C. The upper aqueous phase containing the RNA was carefully transferred to a new RNase-free microtube. RNA was precipitated by adding 0.5 mL of isopropanol, mixing by inversion, and incubating at -20°C for 1 hour. The samples were then centrifuged at 12,000 × g for 10 minutes at 4°C. The supernatant was discarded, and the RNA pellet was washed with 1 mL of 75% ethanol. After vortexing, the sample was centrifuged at 7,500 × g for 5 minutes at 4°C. The supernatant was carefully removed, and the pellet was air-dried for 5-10 minutes. The RNA was resuspended in 20 μL of nuclease-free water by incubating at 60°C for 5 minutes to facilitate dissolution.

RNA concentration and purity were assessed spectrophotometrically. cDNA was synthesized from 1 μg of total

RNA using a commercial kit (Fermentas, USA) according to themanufacturer's instructions. The synthesized cDNA was used as a template for quantitative real-time PCR (qRT-PCR).

The expression levels of renal TNF-α and NF-κB were quantified by qRT-PCR using SYBR Green master mix. Primer sequences for target genes (TNF-α, NF-κB) and the reference gene (GAPDH) were designed based on sequences in the NCBI database and are listed in Table 1. Primer sets were validated prior to use: a standard curve generated from a serial dilution of cDNA was used to calculate primer amplification efficiency, which was confirmed to be between 90% and 110%. Furthermore, melting curve analysis was performed at the end of each run to confirm the specificity of amplification and the absence of primer-dimers.

The relative gene expression was calculated using the 2^(–ΔΔCt) method. The threshold cycle (Ct) value for each target gene was normalized to the Ct value of the GAPDH housekeeping gene from the same sample (ΔCt = Ct_Target – Ct_GAPDH). The ΔΔCt was then calculated by subtracting the ΔCt of the control group (reference) from the ΔCt of each experimental sample (ΔΔCt = ΔCt_Sample – ΔCt_Control). The fold change in gene expression was expressed as 2^(–ΔΔCt). The sequences of the primers used are reported in Table 1.

## Statistical analysis

The normality of data distribution was checked and confirmed using the Shapiro-Wilk test. In order to determine the significance of the difference between the variables of the research groups, one-way analysis of variance and post hoc Tukey's test were used. Pearson correlation was used to consider the relation of genes with HOMA-IR. Mean and standard deviation were used for descriptive data reporting. After collecting the required information, it was analyzed using SPSS version 26 statistical software at a significance level of at least p≥0.05.

# Results

The results of insulin resistance of different study groups are shown in Figure 1. According to the results of one-way ANOVA, diabetes induction and therapeutic interventions caused significant differences between different study groups (F=12.80, p<0.0001). The results of Tukey's post hoc test showed that the DI+HIIT (p=0.0006), DI+RJ (p=0.0011) and DI+HIIT+RJ (p<0.0001) groups showed a significant decrease in HOMA-IR compared to the diabetes control group (Figure 1).

The results of TNF-α and NF-kB gene expression in different research groups are shown in Figure 2 (A & B). According to the results of one-way ANOVA, diabetes induction and therapeutic interventions caused a significant difference between different research groups in TNF-α gene expression (F=136.8, p<0.0001). The results of Tukey's post hoc test showed that the DI+HIIT (p<0.0001), DI+RJ (p<0.0001) and DI+HIIT+RJ (p<0.0001) group

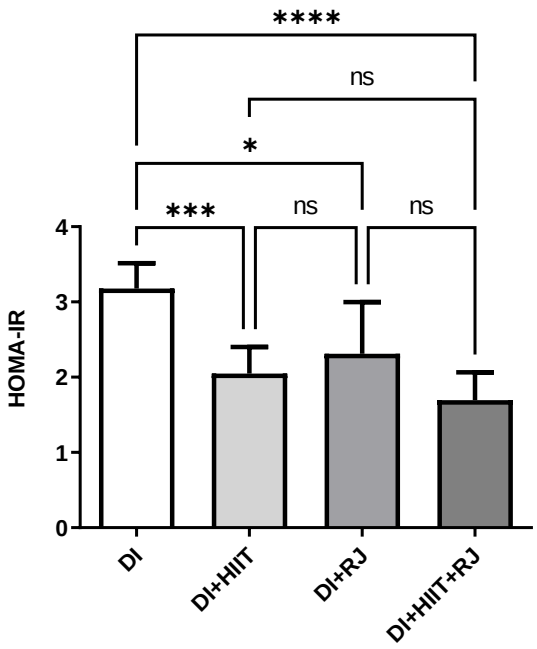


Figure 1. Changes in HOMA-IR at different groups of study. Data are shown as mean and standard deviation. The significance level is (p≥ 0.05). *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. Abbreviations: DI: Diabetes, HIIT: High intensity interval training, RJ: Royal Jelly

Table 1. Primer sequence.

| Gene | sequence | Product lenght | Accession number |
|---|---|---|---|
| Rattus norvegicus glyceraldehyde-3-phosphate dehydrogenase (Gapdh) | F: CAAGTTCAACGGCACAGTCA | 102 nt | NM_017008.4 |
| | R: CCCCATTTGATGTTAGCGGG | | |
| Rattus norvegicus tumor necrosis factor (TNF-a) | F: ATGGGCTCCCTCTCATCAGT | 106 nt | NM_012675.3 |
| | R: GCTTGGTGGTTTGCTACGAC | | |
| Rattus norvegicus RELA proto-oncogene, NF-kB subunit | F: CGTGAGGCTGTTTGGTTTGA | 89 nt | NM_199267.2 |
| | R: TCTGCCCTCCTGACTCTACT | | |

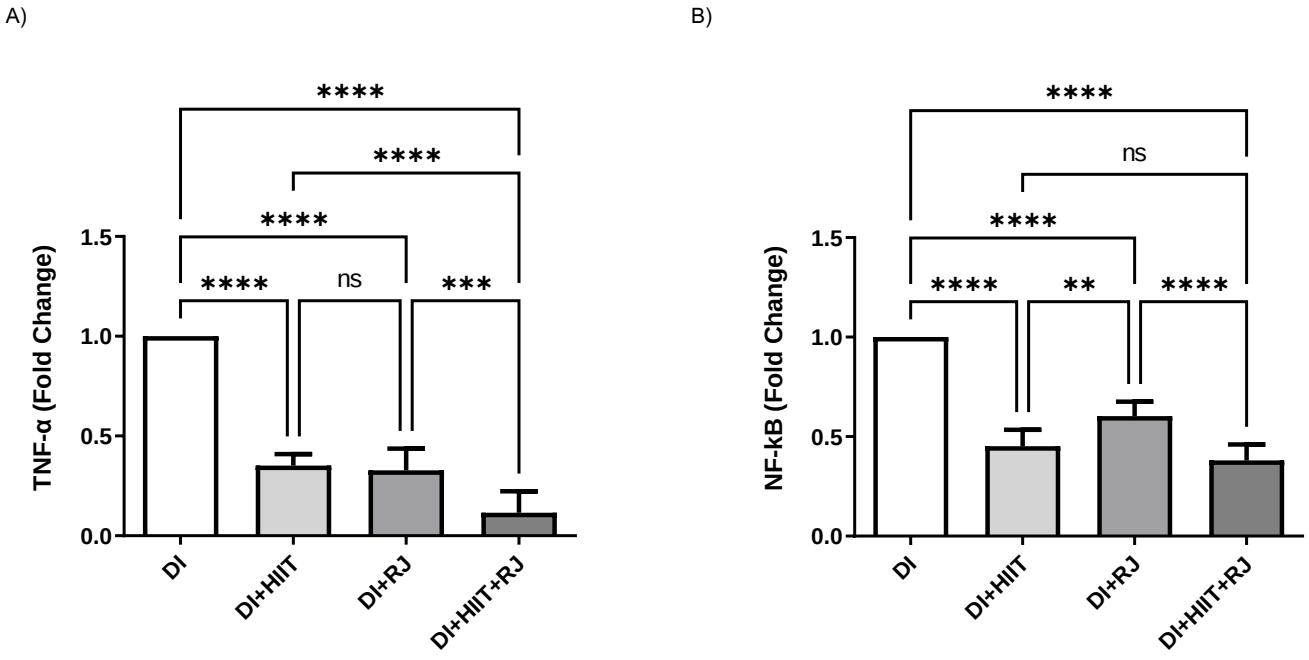


Figure 2. Changes in expression of TNF-α (A) and NF-kB (B) genes at different groups of study. Data are shown as mean and standard deviation. The significance level is (p≥ 0.05). *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. Abbreviations: DI: Diabetes, HIIT: High intensity interval training, RJ: Royal Jelly.

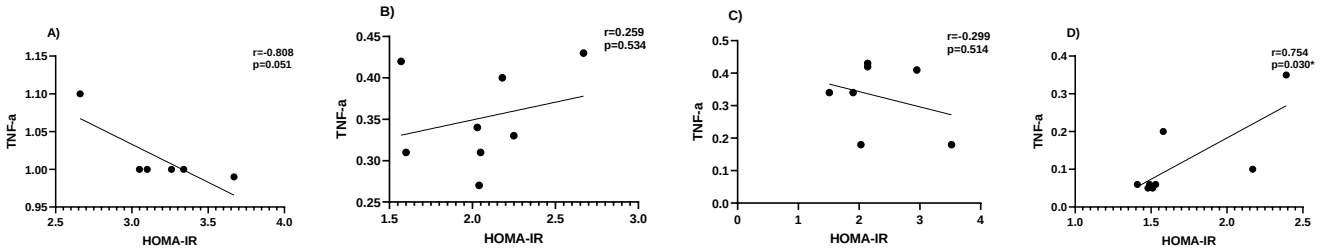


Figure 3. Pearson correlation between HOMA-IR and TNF-α at different groups (A: DI, B: DI+HIIT, C: DI+RJ, D: DI+HIIT+RJ). The data were show as mean ± standard division (p<0.05). Abbreviations: DI: Diabetes, HIIT: High intensity interval training, RJ: Royal Jelly.

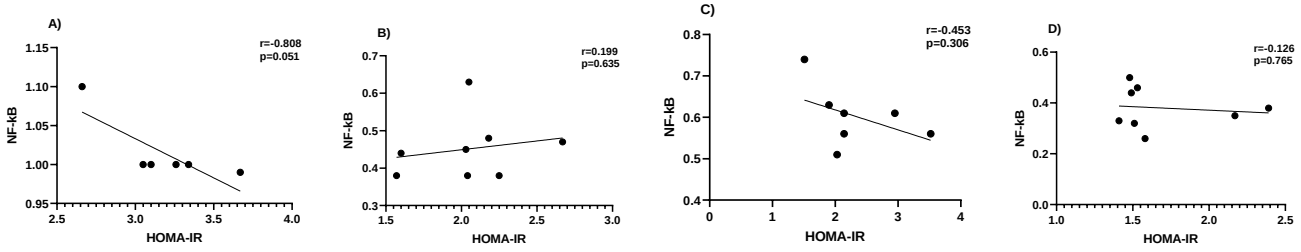


Figure 4. Pearson correlation between HOMA-IR and NF-kB at different groups (A: DI, B: DI+HIIT, C: DI+RJ, D: DI+HIIT+RJ). The data were show as mean ± standard division (p<0.05). Abbreviations: DI: Diabetes, HIIT: High intensity interval training, RJ: Royal Jelly

groups showed a significant decrease in TNF-α gene expression compared to the diabetes control group (Figure 2A). The greatest decrease was related to the combination treatment group.

Also, according to the results of one-way ANOVA, diabetes induction and therapeutic interventions caused a significant difference between different research groups in NF-kB gene expression ($F=99.99$, $p<0.0001$). Tukey's post hoc test results showed that the DI+HIIT ($p<0.0001$), DI+RJ ($p<0.0001$), and DI+HIIT+RJ ($p<0.0001$) groups showed a significant decrease in NF-kB gene expression compared to the diabetic control group (Figure 2B). The greatest decrease was observed in the combination treatment group.

Pearson correlation method was used to examine the relationship between HOMA-IR and TNF-α. The results of Pearson correlation test showed that there was no significant difference between HOMA-IR and TNF-α in different groups except for the combination therapy group ($r=0.754$, $p=0.030$) (Figure 3 A-D).

Pearson correlation method was used to examine the relationship between HOMA-IR and NF-kB. The results of Pearson correlation test showed that there was no significant difference between HOMA-IR and NF-kB in different groups (Figure 4 A-D).

No other significant correlations were detected. These results suggest that the relationship between insulin resistance and inflammation may be complex and group-specific; however, the mechanistic basis for this isolated finding remains unclear and warrants further investigation.

## Discussion

Exercise training acts through multiple mechanisms—anti-inflammatory effects, improved blood glucose control, better vascular function, and direct kidney tissue protection—to slow the progression of diabetic kidney disease and improve kidney outcomes in diabetes. It is considered a safe and effective adjunct to medication and dietary management for DKD patients. Therefore, the aim of the present study was to investigate the effect of 8 weeks of interval training and royal jelly on the expression of renal inflammatory genes and insulin resistance in diabetic rats.

The significant reduction in HOMA-IR observed in the Diabetes+HIIT, Diabetes+RJ, and Diabetes+HIIT+RJ groups compared to the diabetes control group can be attributed to distinct and complementary mechanisms of high intensity interval training (HIIT) and royal jelly (RJ). HIIT improves insulin sensitivity primarily through enhanced activation of key metabolic and insulin signaling pathways, including increased AKT/FOXO1 phosphorylation and GLUT4 expression in skeletal muscle. These molecular changes reduce hepatic gluconeogenesis and facilitate greater glucose uptake by muscle cells. HIIT is also ass-ociated with reductions in systemic inflammation and oxidative stress, which further contribute to improved insulin action and glycemic control in diabetes (Jiménez-Maldonado et al., 2020). Royal jelly, on the other hand, exerts its effects via antioxidant and anti-inflammatory properties, as well as through bioactive compounds with insulin-mimetic activity. RJ supplementation has been shown to increase serum total antioxidant capacity and decrease HOMA-IR in diabetic subjects, likely by mitigating oxidative stress—a key mediator of insulin resistance in diabetes. Additionally, RJ may modulate the expression of genes involved in glucose uptake (e.g., AMPK activation) and reduce circulating retinol-binding protein 4 (RBP4), which is implicated in the development of insulin resistance (Shidfar et al., 2015).

High-intensity interval training (HIIT) and royal jelly (RJ) appear to reduce inflammation in diabetes through interconnected molecular mechanisms involving the downregulation of TNF-α and NF-κB. HIIT attenuates the expression of pro-inflammatory cytokines, particularly TNF-α, by enhancing anti-inflammatory mediators such as IL-10 during muscle contraction. This limits macrophage activation and systemic inflammation, commonly elevated in diabetes. HIIT also reduces NF-κB activation—a central mediator in the inflammatory cascade—through increased antioxidant defense, improved glycemic control, and decreased oxidative stress. The reduction of these inflammatory markers has been linked to the activation of cytoprotective peptides such as Humanin, which suppress oxidative and inflammatory stress in diabetic tissue (Paramita et al., 2022). Royal jelly exerts anti-inflammatory effects through bioactive compounds, notably 10-hydroxy-2-decenoic acid (10-HDA), which suppresses pro-inflammatory cytokine transcription by inhibiting the NF-κB pathway. RJ achieves this by stabilizing IκB, the inhibitor of NF-κB, thereby preventing the nuclear translocation required for TNF-α and other cytokine gene expression. Additionally, RJ upregulates Nrf2, promoting antioxidant defenses and indirectly diminishing NF-κB–mediated inflammation. Experimental studies report significant decreases in TNF-α and NF-κB expression with RJ supplementation in diabetic models (Baptista et al., 2023). The combination of HIIT and RJ may produce additive or synergistic anti-inflammatory effects, as both independently modulate the NF-κB pathway and TNF-α expression, potentially offering superior reduction of inflammatory burden and protection against diabetes-induced tissue damage compared to either intervention alone. This mechanistic insight supports the observed decreases in TNF-α and NF-κB gene expression in the present study and highlights the therapeutic promise of combined lifestyle and nutritional interventions for mitigating inflammation in diabetes (Soltany et al., 2025).

TNF-α, a pro-inflammatory cytokine, has been extensively implicated in the pathogenesis of insulin resistance (IR) and type 2 diabetes. TNF-α interferes with insulin signaling pathways primarily by promoting serine phosphorylation of insulin receptor substrate (IRS) proteins, thereby impairing downstream insulin signaling and glucose uptake in peripheral tissues (Swaroop et al., 2012). This cytokine also exacerbates systemic inflammation and lipolysis, leading to elevated free fatty acids that further impair insulin sensitivity (Swaroop et al., 2012). In the present study, the significant positive correlation observed between HOMA-IR and TNF-α in the combination therapy group (r=0.754, p=0.030) may reflect an association between these parameters. While correlation does not imply causation, this finding is consistent with the known biology wherein elevated TNF-α levels are associated with higher insulin resistance indices in diabetic patients. This interesting association could suggest a potential link between the inflammatory milieu and metabolic dysregulation in this specific treatment group, warranting further investigation. It is plausible that the combination of exercise and royal jelly modulated these pathways in a way that made this correlation detectable, whereas other interventions did not. Taken together, these findings highlight TNF-α as a critical mediator linking inflammation and insulin resistance in diabetes in the established literature, supporting the therapeutic targeting of inflammatory pathways to improve metabolic outcomes.

A potential limitation of this study is that while a placebo (distilled water) was administered to control for the physical stress of gavage, it does not control for potential physiological effects of other components in royal jelly beyond the phenolics of interest. The stress of the gavage procedure itself was, however, accounted for in all groups. Furthermore, the mechanistic pathways discussed, while supported by previous literature, were not directly measured at the protein or phosphoprotein level in this study. The interpretation of the correlation between HOMA-IR and TNF-α remains speculative and should be considered a hypothesis-generating finding for future research. Also, the absence of functional renal data (e.g., serum creatinine, BUN) or histological analysis is a study limitation, and future work is needed to directly link these molecular findings to physiological outcomes in the kidney.

## Conclusion

In conclusion, this study demonstrates that both high-intensity interval training (HIIT) and royal jelly (RJ), individually and in combination, effectively improve insulin resistance and attenuate renal inflammation in diabetic rats. These interventions exert complementary effects through molecular pathways that enhance insulin signaling, reduce oxidative stress, and suppress key inflammatory mediators such as TNF-α and NF-κB. The significant positive correlation between HOMA-IR and TNF-α in the combination therapy group underscores the critical role of inflammation in the progression of insulin resistance and highlights the potential of targeting inflammatory pathways for metabolic and renal protection in diabetes. The additive anti-inflammatory and metabolic benefits observed with combined HIIT and RJ suggest that lifestyle modifications paired with nutritional supplementation may offer a promising therapeutic strategy to mitigate diabetic kidney disease and improve overall metabolic health. These findings support further exploration of integrated exercise and nutritional interventions as safe and effective adjuncts in diabetes management.

## What is already known on this subject?

Insulin resistance, a hallmark of type 2 diabetes and frequently present in kidney disease, worsens renal outcomes through several mechanisms.

## What this study adds?

High-intensity interval training (HIIT) and royal jelly (RJ), individually and in combination, effectively improve insulin resistance and attenuate renal inflammation in diabetic rats.

**Organ Cross-Talk Tips:**

- Its anti-inflammatory effect on the kidneys also improves systemic metabolic status, showing the link between metabolic organs and the kidney.
- The combination produced the greatest reduction in renal inflammation and insulin resistance, illustrating enhanced organ cross talk through simultaneous systemic interventions targeting multiple pathways.

## Acknowledgements

None

## Funding

No sources of funding were sought or awarded for this study.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This study was approved by the Medical Ethics Committee of Azad University of Science and Research under the ethics code IR.IAU.SRB.REC.1402.292.

Informed consent Animal study.

## Author contributions

Conceptualization: M.Zh, F.Gh, S.Sh, H.A.,; Methodology: M.Zh, F.Gh,; Software: S.Sh, H.A,; Validation: S.Sh, H.A,; Formal analysis: M.Zh, F.Gh,; Investigation: S.Sh, H.A,; Resources: S.Sh, H.A,; Data curation: S.Sh, H.A,; Writing - original draft: M.Zh, F.Gh,; Writing - review & editing: M.Zh, F.Gh,; Visualization: S.Sh, H.A,; Supervision: H.A,; Project administration: M.Zh, F.Gh,; Funding acquisition: H.A.

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