

Original Research Article

Effects of quercetin on the expression of insulin resistance-related microRNAs and PTEN/AKT–SOCS3/IRS1 signaling genes in multiple tissues of type 2 diabetic rats

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Abstract

Objective: Quercetin, a natural flavonoid, offers potential benefits for ameliorating insulin resistance and may influence epigenetic mechanisms, including the expression of microRNAs (miRNAs) involved in this pathway. This study aimed to investigate the effects of quercetin on hepatic, renal, cardiac, and skeletal muscle tissues in type 2 diabetic rats by examining the expression of key miRNAs and genes involved in insulin resistance and insulin signaling pathways, as well as the correlations between their expression levels.

Materials and Methods: A diabetic model was induced in rats using high-fat diet and streptozotocin (HFD/STZ), and then, rats were treated with quercetin at a dose of 30 mg/kg/day by oral gavage for 4 weeks beginning at the time of STZ injection.

Results: RT-qPCR assays revealed that quercetin-treated HFD/STZ rats exhibited downregulation of *miR-222*, *miR-21*, *miR-146a*, and *SOCS3* across all tissues, and *miR-133a* in all tissues except the liver. Conversely, there was upregulation of *Akt*, *Glut4*, and *IRS1* in all tissues, *miR-126* in all tissues except the liver, and *PTEN* in all tissues except the liver and muscle tissues. Correlation analysis revealed that the expression patterns of miRNAs show tissue-specific effects: in hepatic and renal tissues, miRNAs correlate with the decreased expression of *AKT*, *GLUT4*, and *IRS1*; whereas in cardiac and muscle tissues, miRNAs correlate with the decreased expression of *SOCS3* and *PTEN*.

Conclusion: In conclusion, our findings demonstrate that quercetin alters the expression profiles of key miRNAs and their targets, specifically through the upregulation of insulin-sensitizing genes (*Akt*, *Glut4*, and *IRS1*) and the downregulation of inhibitory markers (e.g., *miR-21* and *SOCS3*), thereby modulating the molecular landscape of the insulin signaling pathway.

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Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder characterized by a cluster of adverse symptoms including insulin resistance, hyperglycemia, inflammation, and hyperlipidemia (Li et al. 2023). Additionally, T2DM is often associated with obesity and other cardiovascular risk factors, leading to the development of cardiovascular disease (CVD) as the leading cause of morbidity and mortality worldwide (Pan et al. 2023). Understanding the underlying cellular signaling pathways involved in insulin resistance such as the Phosphatase and Tensin Homolog (PTEN)/Protein Kinase B (Akt)/Glucose Transporter Type 4 (Glut4) pathway and the Suppressor of Cytokine Signaling 3 (SOCS3)/Insulin Receptor Substrate-1 (IRS1) pathway can significantly enhance the early identification of individuals at risk for complications (Le et al. 2023). These pathways are influenced by a variety of factors including genetic predisposition, dietary habits, physical activity, and environmental exposures (Fazakerley et al. 2023). The progression of T2DM involves a complex interplay between multiple organ systems including the liver, skeletal muscle, adipose tissue, gastrointestinal tract, pancreas, brain, and kidneys (DiCorpo et al. 2022).

Effective therapeutic strategies that utilize natural compounds found in fruits and vegetables to target these critical signaling pathways hold promise for preventing insulin resistance and T2DM (Wu and Wang 2023). Among these natural compounds, quercetin (3,5,7,3',4'-pentahydroxyflavone) is one of the most abundant flavonols in nature and is recognized as a potent dietary antioxidant (Yi et al. 2021a). Several animal studies have demonstrated that long-term consumption of quercetin reduces blood glucose levels and protects the morphology of pancreatic beta cells in streptozotocin (STZ)-induced diabetic rats (Azeem et al. 2023). Additionally, quercetin exhibits

anti-diabetic properties in high-fat diet (HFD) and STZ-induced type 2 diabetic mice. It has been proposed that quercetin may improve insulin resistance by modulating the expression of various genes involved in cellular pathways (Ansari et al. 2022).

MicroRNAs (miRNAs) are a group of non-coding, single-stranded RNA molecules, typically 20-25 nucleotides in length, that have emerged as critical factors in the pathogenesis of diabetes (He et al. 2024). These biological biomarkers regulate gene expression at the post-transcriptional level by binding to the 3' untranslated region of target mRNA sequences (Mao et al. 2020). Various miRNAs, including *miR-222*, *miR-21*, *miR-146a*, *miR-133a*, and *miR-126*, have been associated with pathogenic conditions and insulin resistance. Identification of differential expression patterns of miRNAs in metabolic disorders may enable early diagnosis and targeted therapeutic interventions (He et al. 2024; Serik et al. 2023).

Recent studies have shown that *miR-222* whose expression is observed in metabolic organs such as the liver, skeletal muscle, heart, and brain contributes to pancreatic development, regulation of insulin resistance, and onset of diabetes complications through immunological and inflammatory responses (Bibiloni et al. 2023). Similarly, *miR-21* plays a crucial role in regulating pancreatic beta cells, insulin secretion, and controlling molecular signaling pathways involved in diabetes pathogenesis (Chen et al. 2024). Additionally, *miR-146a*, a negative regulator of inflammation, has been implicated in the progression of both type 1 and type 2 diabetes, while *miR-133a* has been demonstrated to modulate insulin resistance and glucose homeostasis in skeletal muscle (da Silva Rosa et al. 2016; Ghaffari et al. 2023).

The role of miRNAs in diabetes is not limited to their influence on insulin secretion and sensitivity. MicroRNAs have

also been shown to be involved in the development of diabetes-related complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy (Bielska et al. 2021). For instance, *miR-126* is essential for maintaining vascular integrity and endothelial function, and its dysregulation has been associated with the development of diabetic microvascular complications (Elham and Javad 2017). The complex interplay between microRNAs and their target genes in the context of diabetes and its complications necessitates a comprehensive understanding of the underlying mechanisms (Ledesma-Pacheco et al. 2023).

The expression of *miR-146a* has been reported to disrupt the insulin signaling pathway during HFD feeding (Alipoor et al. 2017). Additionally, *miR-133a*, a member of the MyomiR group of muscle-specific miRNAs, is associated with diabetic cardiovascular and cardiac pathological conditions that can lead to heart failure (Feng et al. 2010). Overexpression of *miR-126* in hepatocytes has been observed to cause a substantial reduction in IRS-1 protein expression, resulting in impaired insulin signaling (Pishavar and Behravan 2017).

Given the significant impact of quercetin on signaling pathways and the expression of miRNAs involved in insulin resistance, this study aims to investigate the effects of quercetin on the expression of *miR-222*, *miR-21*, *miR-146a*, *miR-133a*, *miR-126*, *PTEN*, *Akt*, *Glut4*, *SOCS3*, and *IRS1*. The goal is to enhance the management of insulin resistance. Additionally, this study analyzes the correlations between miRNA expressions and gene expressions to further elucidate the mechanisms by which quercetin may ameliorate insulin resistance.

Materials and Methods

Experimental animals and study design

The Institutional Animal Ethics Committee (IAEC) of Shahid Sadoughi University of Medical Sciences of Yazd (Yazd, Iran) approved all of the study's experiments in accordance with the National Institute of Health's guidelines for the use and care of laboratory animals (NIH Publication No. 85–23, revised in 1996) (Clark et al. 1997). The Laboratory Animals Research Center (Pasteur Institute, Tehran, Iran) provided 30 mature male Wistar rats, weighing between 200 and 250 g. The animals were given two weeks to get used to the lab setting before the experiment started. All of the study rats were given chow meal (Pars Dam Co., Tehran, Iran) and unlimited water throughout the investigation. They were housed in stainless steel cages and kept in conventional light-dark cycle (12 hr of light and 12 hr of dark) with a temperature adjusted between 22 and 24 degrees Celsius and 50 ± 5% humidity. The experimental design and study timeline are described in Figure 1. All the experimental animals, except the control group, were fed with high fat diet (HFD) *ad libitum*. Given that the composition and preparation of HFD were according to the previous trials (Reed et al. 2000). After four weeks of the prescribed diet, the rats treated by HFD were injected intraperitoneally (IP) with a low dose of STZ (35 mg/kg), while other rats were intraperitoneally given the equivalent volume of citrate buffer (pH 4.5; 1 ml/kg body weight). Subsequently seven days after STZ injection, the fasted rats (10–12 hr) were given glucose (2 g/kg) via gastric intubation and blood glucose concentration was evaluated after 2 hr in order to confirm the induction of T2D. Rats having blood glucose higher than 200 mg/dl were considered diabetic. Quercetin used in this study was purchased as a powder from Sigma Company with purity of ≥95% purity. Quercetin 30 (mg/kg/day) was

prepared as a suspension in carboxymethylcellulose (CMC 0.5%, w/v).

In the present experimental interventional study, Animals were randomly assigned to three equal groups (n = 10 per group):

- Control group (Control): non-diabetic rats fed a normal diet for 8 weeks
- T2D control group (HFD/STZ): diabetic rats fed HFD for 8 weeks without treatment
- Quercetin group (HFD/STZ) +Q: diabetic rats treated with quercetin (30 mg/kg/day, oral gavage) for 4 weeks beginning at the time of STZ injection

It should be mentioned that the dose of quercetin used in the current study was

based on the previous animal investigations (Dhanya et al. 2017).

Measurement of oral glucose tolerance test (OGTT)

The day before rats were sacrificed under general anesthesia by ketamine (90 mg/kg) and xylazine (10 mg/kg), to assess the OGTT, HFD–STZ–diabetic rats were kept fasting overnight, and then, their blood glucose response to the oral administration of a solution of 20% glucose (2 g/kg of body weight) dissolved in water via gavage was determined. Blood samples were collected from the tail vein at 0, 30, 60, and 120 min post-administration. Glucose levels were then measured from the capillary blood using a handheld glucometer. Animals were not anesthetized for this procedure.

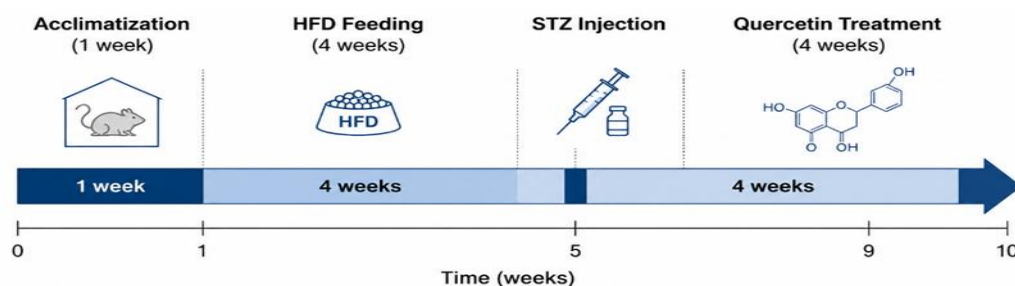


Figure 1. The experimental design and study timeline

RNA separation and the RT-qPCR test

Rat liver, kidneys, heart, and skeletal muscles tissues were subjected to FavorPrep (Total tissue mini-RNA kit) to extract total RNA and underwent reverse transcription to complementary DNA (Yektatajhizazma, Tehran, Iran). After that, real-time reverse transcription PCR was performed out using the master mix Super SYBR Green qPCR (Yektatajhiz, Tehran, Iran). Oligonucleotide primer sequences used for real-time PCR are shown in Table 1. In addition, the cDNA synthesis for miRNAs was carried out in two steps according to the protocol of BONmiR High Sensitivity MicroRNA 1st Strand cDNA Synthesis Kit, Iran. The internal controls for miRNAs and mRNA were *SNORD* and β -actin mRNA, respectively. The

BONMIR Company (Iran) provided the *SNORD* and miRNA primer sequences. Relative gene levels are expressed as ratios to the control, and fold changes were calculated using the $2^{-\Delta\Delta C_t}$ technique.

Statistical analysis

The software GraphPad Prism 6.01 (La Jolla, CA, USA) was used to conduct the statistical analysis. The mean differences for miRNAs and gene expression were compared between the research groups using a one-way ANOVA and Tukey multiple range post-hoc tests. Using the Spearman correlation test in GraphPad Prism, relationships between microRNAs and gene expression were determined. At $p \leq 0.05$, all of the differences were deemed significant.

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Table 1. Sequences of primers for real-time PCR.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product Size (bp)
<i>SOCS3</i>	GGGACCAAGAACCTACGC	GCTGCTCCTGAACCTCAAA	188
<i>IRS1</i>	TGGATGCAAGTGGATGACTC	CGGAGGATTGTTGAGATGGT	170
<i>PTEN</i>	ATACCAGGACCAGAGGAAACC	TTGTCATTATCCGCACGCTC	158
<i>AKT</i>	GTGGCAAGATGTGTATGAG	CTGGCTGAGTAGGAGAAC	172
<i>GLUT4</i>	ACAATGTCTTGGCTGTGCTG	TCCCACATACATAGGCACCA	147
<i>β-actin</i>	CGTTGACATCCGTAAAGACCTC	AGCCACCAATCCACACAGA	172

Results

Table 2 presents the blood glucose levels measured during the OGTT at baseline (0 min) and at 30, 60, and 120 min following the oral glucose load. In the HFD/STZ group, the glucose level was steadily elevated compared to the control group, while quercetin (Q) treatment significantly declined glucose levels ($p < 0.05$). The OGTT showed that glucose tolerance decreased in HFD/STZ diabetic rats when comparing with the control group ($p < 0.05$), while this impairment was improved in quercetin-treated diabetic rats ($p < 0.05$).

Gene expression

Liver tissue

In the liver, the HFD/STZ group demonstrated significant dysregulation in miRNA expression, with elevated levels of *miR-222*, *miR-21*, and *miR-146a* and reduced levels of *miR-126* and *miR-133a* compared to the control group ($p < 0.05$).

These miRNA alterations are consistent with hepatic insulin resistance and inflammatory responses. Quercetin treatment significantly reduced *miR-222*, *miR-21*, and *miR-146a* expression while increasing *miR-126* and *miR-133a* expression levels, suggesting an improvement in hepatic insulin sensitivity and inflammatory control.

The expression of *PTEN*, *Akt*, and *GLUT4* was significantly downregulated in the HFD/STZ group, while *SOCS3* expression was elevated, indicating a disrupted insulin signaling pathway. Quercetin administration normalized these markers, with a significant upregulation of *PTEN*, *Akt*, and *GLUT4* ($p < 0.05$) and a concurrent reduction in *SOCS3* expression. Furthermore, *IRS1* levels which were significantly reduced in the HFD/STZ group, were restored following quercetin treatment, underscoring its potential in mitigating hepatic insulin resistance and restoring glucose homeostasis (Figure 2).

Table 2. OGTT findings

Time	Control	HFD/STZ	(HFD/STZ) +Q
0 min	76.57 ± 16.39	112.42 ± 53.23#	88.17 ± 21.83*
30 min	102.42 ± 8.94	298.21 ± 137.81#	184.72 ± 35.09*
60 min	129.71 ± 13.22	311.64 ± 179.70#	182.86 ± 76.74*
120 min	108.85 ± 6.96	266.81 ± 184.12#	104.01 ± 13.97*

The data are shown as mean ± SD. # $p < 0.05$ in comparison to the control group; * $p < 0.05$ in comparison to the HFD/STZ group. OGTT, oral glucose tolerance test; HFD, High fat diet; STZ, Streptozotocine; Q, Quercetin.

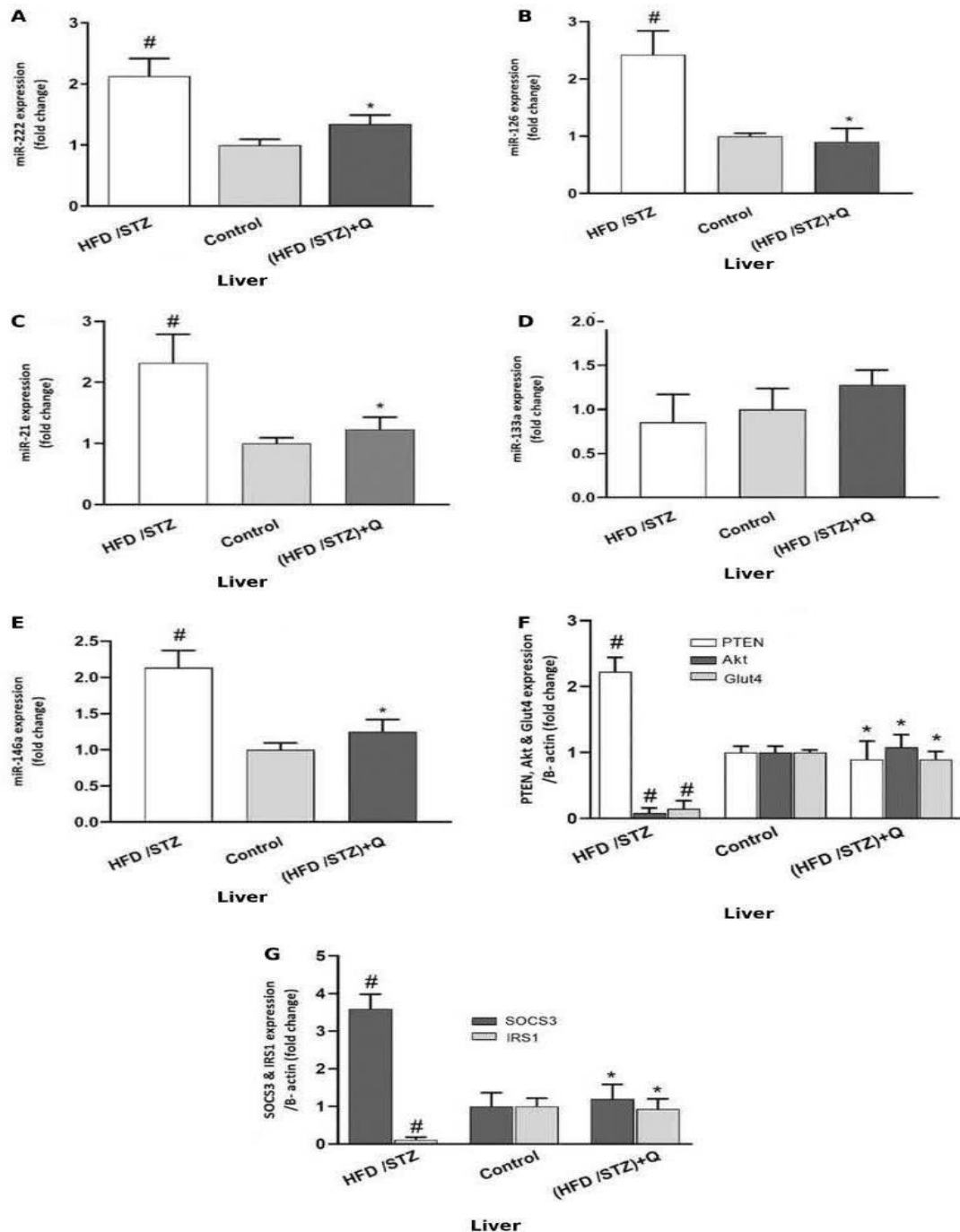


Figure 2. Gene expression of *miR-222* (A), *miR-126* (B), *miR-21* (C), *miR-133a* (D), *miR-146a* (E), *PTEN*, *Akt*, *GLUT4* (F), and *SOCS3* and *IRS1* (G) in liver tissues across the three studied groups (Control, HFD/STZ, HFD/STZ+Q). # $p < 0.05$ compared to the control group. * $p < 0.05$ compared to the HFD/STZ group.

Renal tissue

In renal tissues, the HFD/STZ group exhibited substantial dysregulation of miRNA expression, characterized by elevated *miR-222*, *miR-21*, and *miR-146a* levels, and reduced *miR-126* and *miR-133a* expression compared to the control group ($p < 0.05$). Quercetin treatment significantly

reversed these trends, with reduced *miR-222*, *miR-21*, and *miR-146a* expression and increased *miR-126* and *miR-133a* expression, indicating improved renal insulin sensitivity and reduced inflammation.

Similarly, the HFD/STZ group showed reduced *PTEN*, *Akt*, and *GLUT4* expression, alongside elevated *SOCS3*

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levels and reduced *IRS1* levels, reflecting impaired renal insulin signaling. Quercetin treatment significantly restored *PTEN*, *Akt*, and *GLUT4* expression, while *SOCS3* levels were markedly decreased and *IRS1*

expression was restored ($p < 0.05$). These findings underscore the protective role of quercetin against renal complications associated with diabetes. (Figure 3).

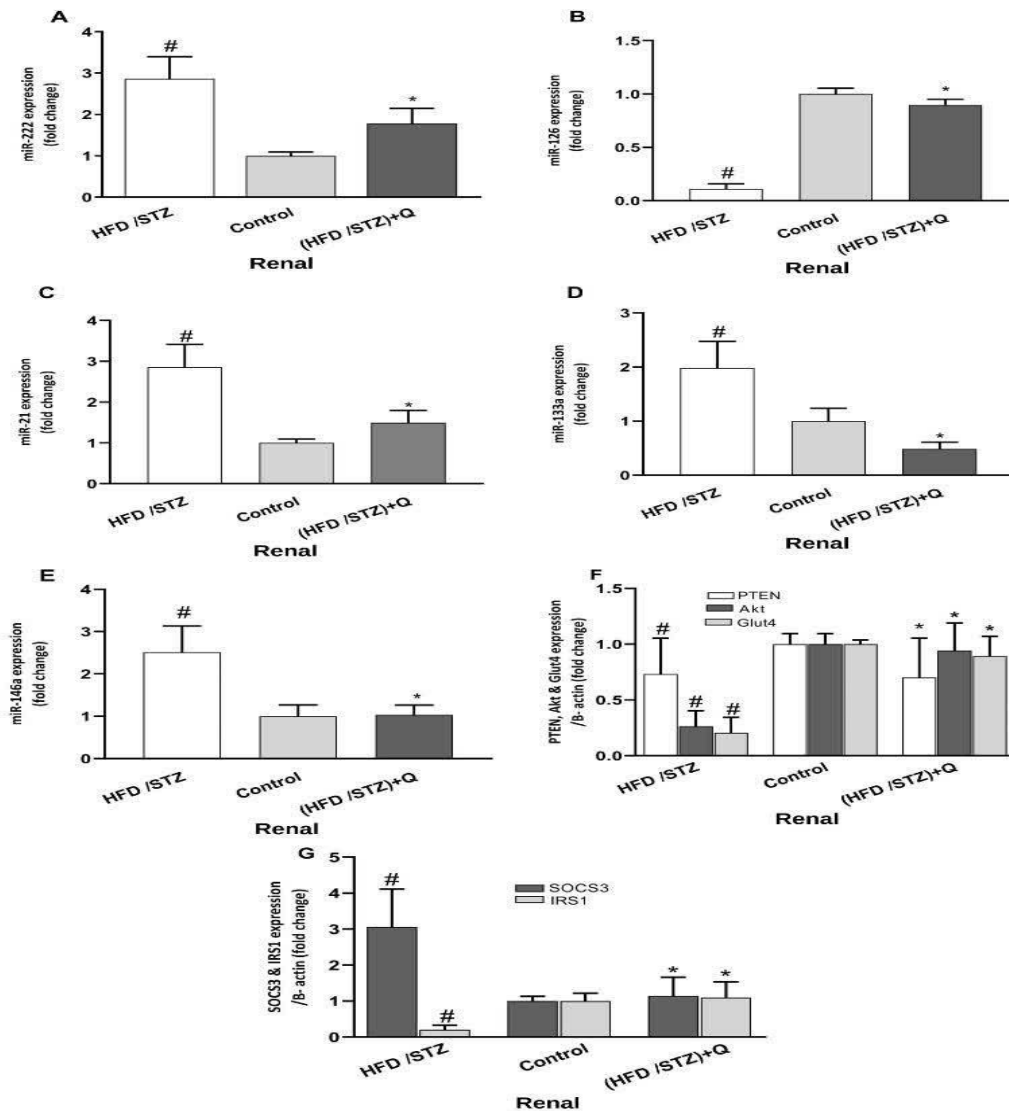


Figure 3. Gene expression of *miR-222* (A), *miR-126* (B), *miR-21* (C), *miR-133a* (D), *miR-146a* (E), *PTEN*, *Akt*, *GLUT4* (F), and *SOCS3* and *IRS1* (G) in renal tissues across the three studied groups (Control, HFD/STZ, HFD/STZ+Q). # $p < 0.05$ compared to the control group. * $p < 0.05$ compared to the HFD/STZ group.

Cardiac tissue

In heart tissues, the expression levels of miRNAs and key signaling genes showed significant changes across the studied groups. The HFD/STZ group exhibited marked dysregulation of miRNAs, including upregulation of *miR-222*, *miR-146a*, and *miR-133a*, and downregulation of *miR-126* and *miR-21*, compared to the

control group. These changes highlight the detrimental effects of insulin resistance on cardiac tissues. Quercetin administration effectively normalized these miRNA levels, bringing them closer to the control group. Specifically, *miR-222* expression was significantly reduced ($p < 0.05$), while *miR-126* and *miR-21* levels were elevated in the HFD/STZ+Q group compared to the untreated diabetic group ($p < 0.05$).

At the gene level, the HFD/STZ group showed significant downregulation of *PTEN*, *Akt*, and *GLUT4*, alongside upregulation of *SOCS3* and reduced *IRS1* levels, reflecting impaired glucose uptake and signaling in the heart. Quercetin treatment significantly upregulated *PTEN*,

Akt, and *GLUT4* expression, restoring glucose metabolism pathways. Additionally, *SOCS3* expression was significantly reduced, and *IRS1* levels were restored, indicating improved insulin signaling in the cardiac tissue of diabetic rats treated with quercetin (Figure 4).

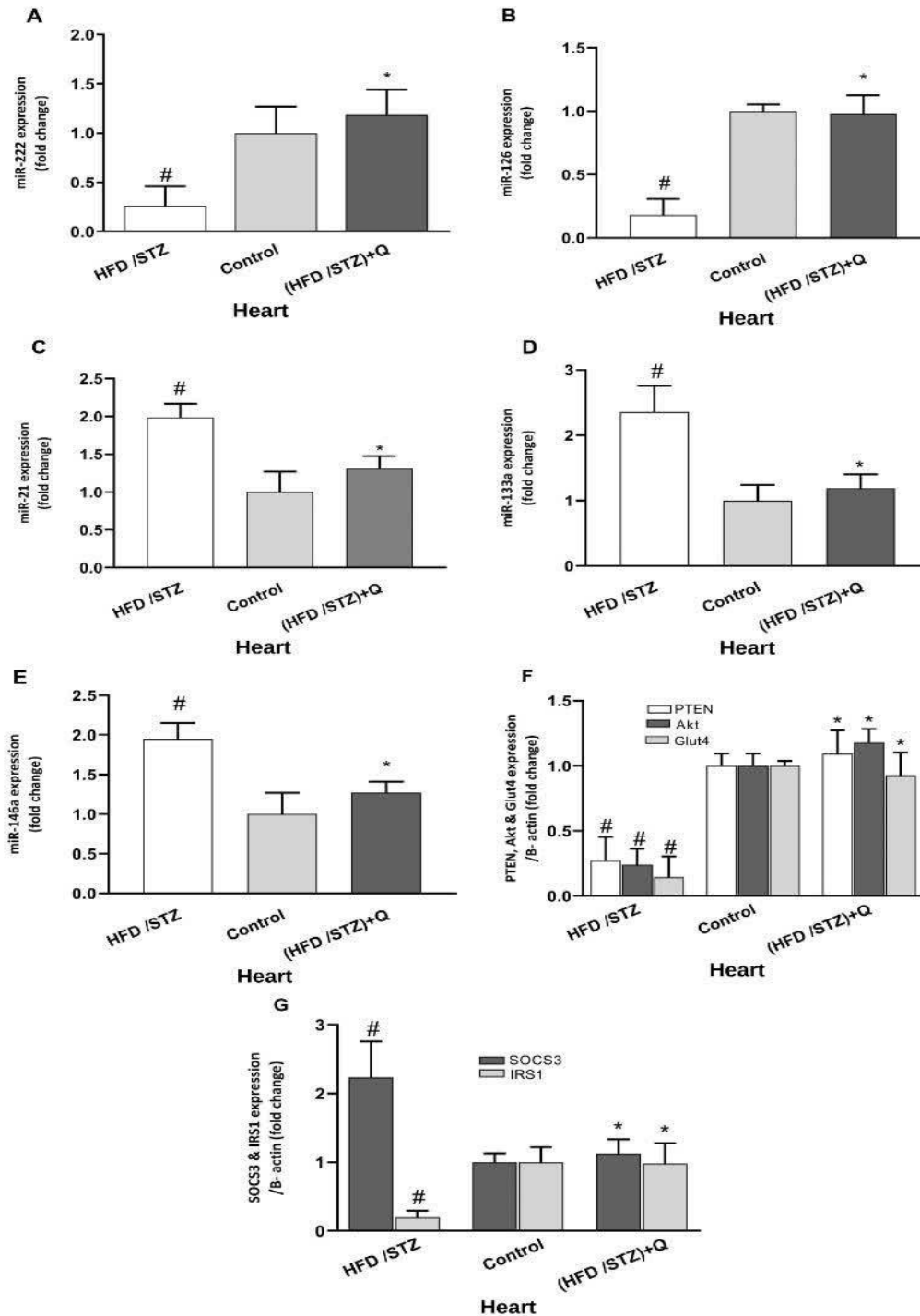


Figure 4. Gene expression of *miR-222* (A), *miR-126* (B), *miR-21* (C), *miR-133a* (D), *miR-146a* (E), *PTEN*, *Akt*, *GLUT4* (F), *SOCS3* and *IRS1* (G) in heart tissues across the three studied groups (Control, HFD/STZ, HFD/STZ+Q). # $p < 0.05$ compared to the control group. * $p < 0.05$ compared to the HFD/STZ group. HFD/STZ: high-fat diet/streptozotocin; Q: Quercetin.

Skeletal muscle tissue

Skeletal muscle tissues showed profound miRNA dysregulation in the HFD/STZ group, with elevated levels of *miR-222*, *miR-21*, and *miR-146a*, and reduced levels of *miR-126* and *miR-133a* ($p < 0.05$). These changes are indicative of impaired glucose uptake and utilization in muscle tissues. Quercetin treatment effectively normalized these miRNA levels, particularly reducing *miR-222* and *miR-146a* expression while enhancing *miR-126* and *miR-133a* expression compared to the untreated diabetic group ($p < 0.05$).

At the gene expression level, the HFD/STZ group displayed significant downregulation of *PTEN*, *Akt*, and *GLUT4* and upregulation of *SOCS3*, indicating insulin signaling impairment. Quercetin administration significantly improved *PTEN*, *Akt*, and *GLUT4* expression, enhancing glucose transport and metabolism in muscle tissues ($p < 0.05$). *SOCS3* expression was notably reduced, and *IRS1* levels were restored, emphasizing the role of quercetin in reversing skeletal muscle insulin resistance (Figure 5).

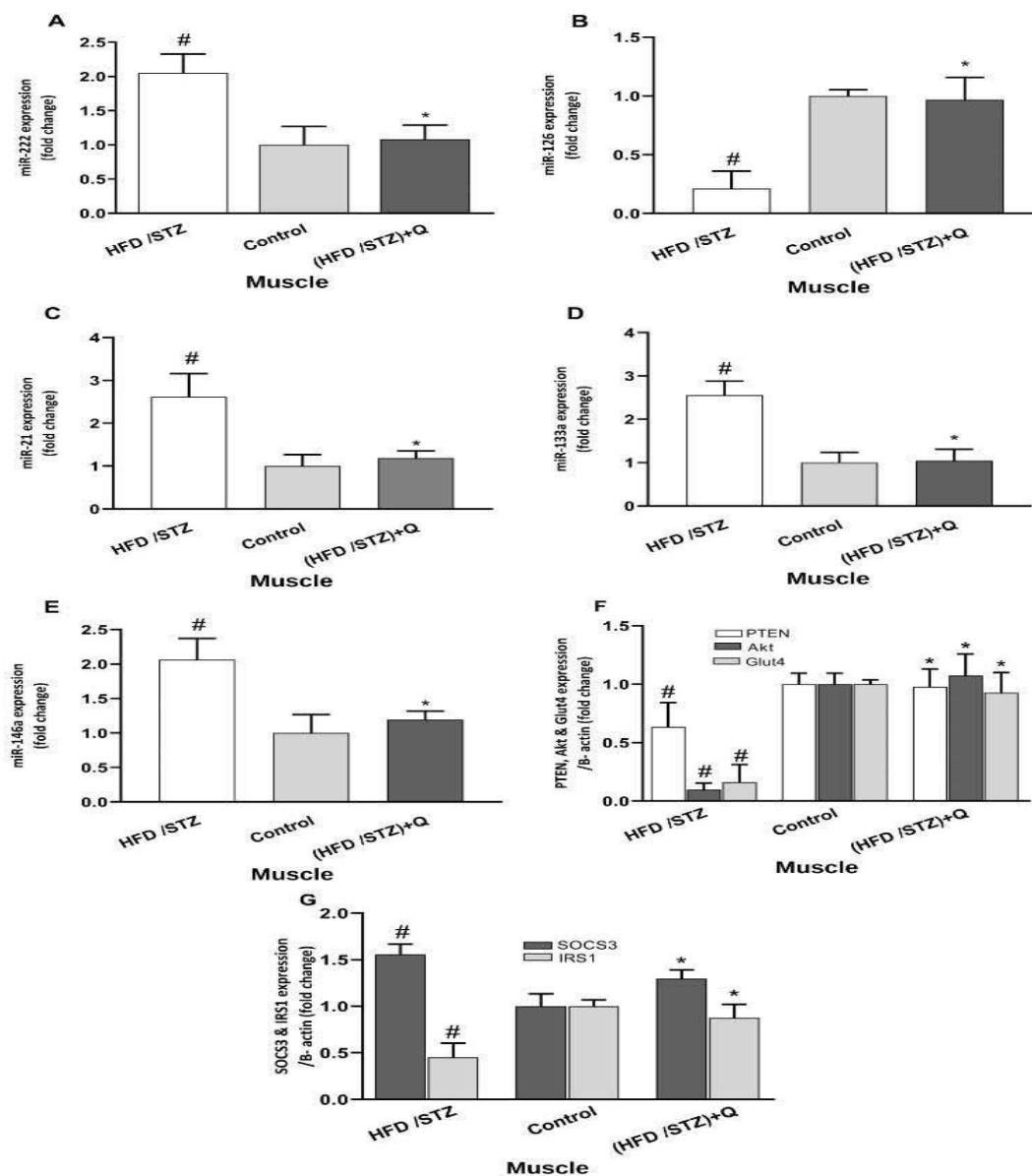


Figure 5. Gene expression of *miR-222* (A), *miR-126* (B), *miR-21* (C), *miR-133a* (D), *miR-146a* (E), *PTEN*, *Akt*, *GLUT4* (F), and *SOCS3* and *IRS1* (G) in muscle tissues across the three studied groups (Control, HFD/STZ, HFD/STZ+Q). # $p < 0.05$ compared to the control group. * $p < 0.05$ compared to the HFD/STZ group.

Correlation analysis

The Spearman correlation analysis provided additional insights into the relationships between miRNA expression and key insulin resistance-related genes across tissues (Figure 6). In the liver panel (A), several miRNAs show moderate to strong negative correlations with insulin-signaling components like *AKT*, *GLUT4*, and *IRS1*, suggesting that higher miRNA expression may reduce insulin-sensitizing gene expression. *PTEN* correlated positively with some miRNAs and negatively with others, indicating a complex regulatory network in the liver. In the kidney (B), correlations are generally weaker, with many values near zero, implying greater biological variability. Selected miRNAs show negative

correlations with *GLUT4* or *IRS1*, which may reflect tissue-specific targeting. In cardiac tissue (C), strong positive correlations between miRNAs and *AKT* or *GLUT4* suggest co-regulation of expression and support insulin-responsive glucose uptake. Negative correlations with *SOCS3* or *PTEN* indicate that higher miRNA levels may enhance insulin signaling in the heart. In skeletal muscle (D), significant correlations with *GLUT4/IRS1*, along with negative correlations with *SOCS3* and *PTEN*, suggest that miRNAs promote insulin sensitivity by supporting effector genes. More coherent correlations in muscle than in kidney highlight the organized miRNA regulation of insulin signaling, which is crucial for systemic glucose homeostasis.

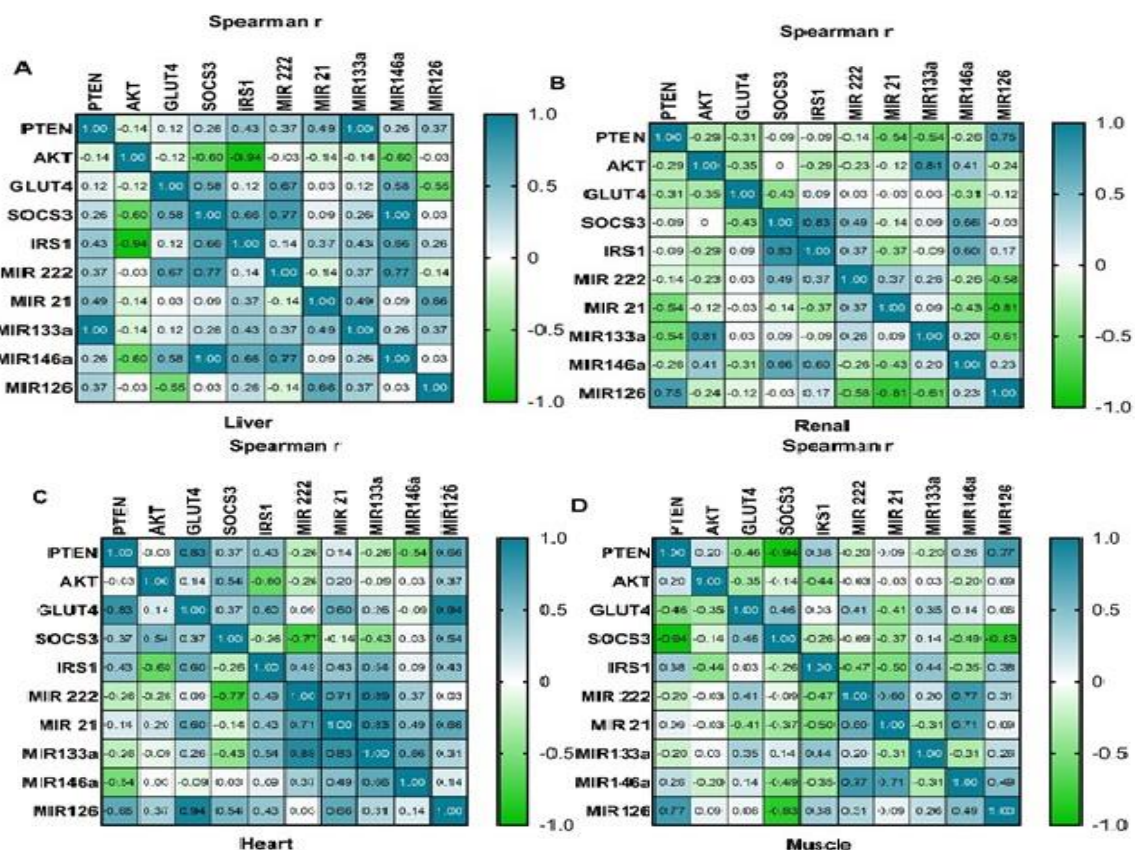


Figure 6. Spearman correlation matrices depicting the associations between miRNA expression levels and key insulin resistance signaling genes. The figure displays pairwise correlation coefficients (Spearman's r) between specific miRNAs (*miR-222*, *miR-126*, *miR-21*, *miR-133a*, and *miR-146a*) and genes involved in the insulin signaling pathway (*PTEN*, *Akt*, *GLUT4*, *SOCS3*, and *IRS1*) across four distinct tissue types: (A) Liver, (B) Renal tissue, (C) Heart, and (D) Muscle. Color intensities represent the strength and direction of the correlation, with blue and green gradients indicating positive and negative correlations, respectively. These data illustrate the tissue-specific regulatory relationships between the analyzed miRNAs and the molecular components of insulin resistance.

Discussion

This study provides compelling evidence for the therapeutic potential of quercetin in mitigating insulin resistance and associated complications in multiple tissues of diabetic rats. The findings contribute significantly to our understanding of the molecular mechanisms underlying quercetin beneficial effects, particularly through the modulation of miRNA expression and key insulin signaling pathways.

Our results demonstrate that quercetin treatment effectively normalized the expression of several miRNAs (*miR-222*, *miR-21*, *miR-146a*, *miR-126*, and *miR-133a*) and insulin signaling genes (*PTEN*, *Akt*, *GLUT4*, *SOCS3*, and *IRS1*) in liver, renal, cardiac, and skeletal muscle tissues of diabetic rats. The observed upregulation of *AKT* transcripts in our study is consistent with previous reports indicating that quercetin modulates key molecular targets involved in insulin signaling, such as *AKT*, *AMPK*, and *NRF2* (Yan et al. 2023).

Quercetin has been shown to exert anti-diabetic, anti-oxidant, and anti-inflammatory properties in various experimental models. Our findings are consistent with previous reports demonstrating the beneficial effects of quercetin on hyperglycemia, dyslipidemia, and oxidative stress in diabetes (Mosad et al. 2024). Quercetin has also been shown to attenuate inflammation and improve insulin sensitivity in various tissues, which may contribute to its protective effects against diabetic complications (Aghababaei and Hadidi 2023).

Our findings align with recent studies highlighting the role of miRNAs in diabetes pathogenesis and the potential of natural compounds in modulating these molecular targets. For instance, Wang et al. reported similar alterations in *miR-222* and *miR-126* expression in diabetic models, corroborating our observations (Wang et al. 2014). However, our study extends these findings by demonstrating quercetin ability to reverse these alterations across multiple

tissues simultaneously. The observed upregulation of *GLUT4* and *Akt* expression following quercetin treatment is consistent with the work, reported enhanced glucose uptake in skeletal muscle cells treated with quercetin (Dhanya et al. 2017). Our study provides evidence supporting these *in vitro* findings, strengthening the case for quercetin insulin-sensitizing effects.

The normalization of miRNA expression by quercetin, particularly the reduction in *miR-222* and increase in *miR-126*, likely contributes to improved insulin sensitivity. *miR-222* has been shown to negatively regulate *GLUT4* expression, while *miR-126* enhances insulin signaling by targeting *PI3K* regulatory subunit p85 β (Bibiloni et al. 2023; Esteves et al. 2017). These findings suggest that quercetin may ameliorate insulin resistance, at least in part, by modulating the expression of these key genes (Dhanya et al. 2017).

In addition to miRNA regulation, quercetin treatment significantly upregulated the expression of insulin signaling genes, such as *Akt* and *GLUT4*, in multiple target tissues. These changes may enhance glucose uptake and utilization, thereby improving glycemic control (Wang et al. 2020). Moreover, the anti-inflammatory properties of quercetin, evident from the reduced levels of inflammatory markers such as tumor necrosis factor-alpha, interleukin-6, and C-reactive protein, may also contribute to its beneficial effects on insulin sensitivity and diabetic complications (Aghababaei and Hadidi 2023; Yi et al. 2021b).

PTEN positive correlation with *miR-126* across multiple tissues aligns with previous findings that *miR-126* plays a protective role in vascular health by regulating *PTEN*, thereby contributing to endothelial function in diabetes. This interaction also supports *miR-126* established role in enhancing insulin sensitivity through the *PI3K/AKT* pathway (Xu et al. 2015).

In the liver, *SOCS3* positive correlation with *IRS1* and *miR-21* mirrors prior

research indicating that *SOCS3* modulates *IRS1* signaling in insulin resistance by suppressing insulin receptor phosphorylation (Xu et al. 2020). Additionally, *miR-21* has been implicated in upregulating *SOCS3* expression, thereby contributing to inflammatory pathways in diabetes (Li and Zeng 2020).

The positive association of *miR-126* and *miR-133a* with *GLUT4* in muscle tissue is consistent with the roles of these miRNAs in promoting glucose uptake. Specifically, *miR-133a* has been shown to enhance *GLUT4* expression, thereby improving insulin sensitivity in skeletal muscle (Mitchelson and Qin 2015).

Furthermore, the muscle-specific correlation of *miR-222* with *AKT* aligns with studies suggesting that *miR-222* modulates *AKT* activity, which is crucial for maintaining insulin-mediated glucose uptake and preventing metabolic dysfunction in diabetes (Li et al. 2020). Inverse correlation between *miR-146a* and *SOCS3* in renal tissue may reflect *miR-146a* role in mitigating inflammation and insulin resistance by downregulating the pro-inflammatory *SOCS3* (de Carvalho Nunes et al. 2020).

The concurrent upregulation of *PTEN*, *Akt*, and *GLUT4*, along with the downregulation of *SOCS3*, suggests that quercetin enhances insulin signaling through multiple mechanisms. Interestingly, the tissue-specific variations in miRNA responses to quercetin treatment highlight the complex nature of insulin resistance and the potential for targeted therapeutic approaches. For instance, the more pronounced effect on *miR-133a* in cardiac tissue compared to other tissues may reflect tissue-specific roles of this miRNA in glucose metabolism (Khodarahmi et al. 2019; M S and C D 2017; Mehta et al. 2017; Tang et al. 2022).

This study highlights quercetin potential as a multi-tissue protective agent in diabetes, modulating miRNA expression and key insulin signaling pathways. While the findings are promising, limitations

include the use of a rat model, short study duration, and a focus on specific miRNAs and genes. Future research should explore genome-wide miRNA effects, synergistic therapies, and clinical trials to confirm efficacy in humans.

The findings suggest that quercetin may exert beneficial effects in experimental diabetes, potentially through miRNA-mediated modulation of insulin signaling pathways in a tissue-dependent manner. In cardiac and skeletal muscle, miRNAs appear to promote insulin sensitivity by suppressing *PTEN* and *SOCS3* while supporting *AKT*, *GLUT4*, and *IRS1*, whereas their roles in liver and kidney are more complex and context-dependent. These tissue-specific patterns indicate that miRNA function cannot be assumed uniform across tissues. Collectively, these results may inform future research into miRNA-targeted strategies for diabetes management, though further mechanistic studies are warranted before therapeutic conclusions can be drawn.

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Conflicts of interest

The authors have declared that there is no conflict of interest.

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Ethical Considerations

The Animal Care Ethic Committee approved the study protocol and Ethic clearance was issued for the study by the Shahid Sadoughi University of Medical Sciences and Health Services (Approval ID: IR.SSU.MEDICINE.REC.1397.030). The rats were housed, maintained and euthanized in accordance to the relevant international rules and recommendations.

Code of Ethics

IR.SSU.MEDICINE.REC.1397.030

Authors' Contributions

A.M. conceived and designed the study, supervised the experimental work, and contributed to manuscript preparation. **M.Y., F.F.** performed the animal experiments, biochemical analyses, and data collection. **A.Kh.** carried out microRNA extraction and qRT-PCR studies. **F.D., M.E.R.** analyzed the data and performed statistical evaluation. **A.Kh, N.P.M.** contributed to the interpretation of signaling pathway data and assisted in figure preparation. **M.S.** wrote the first draft of the manuscript. All authors read, revised, and approved the final version of the manuscript.

Abbreviations

T2DM: Type 2 diabetes mellitus; miRNAs: microRNAs; HFD/STZ: High-Fat Diet/Streptozotocin; Q: Quercetin; CVD: Cardiovascular Disease; PTEN: Phosphatase and Tensin Homolog; Akt: Protein Kinase B; Glut4: Glucose Transporter Type 4; SOCS3: Suppressor of Cytokine Signaling 3; IRS1: Insulin Receptor Substrate-1; OGTT: Oral Glucose Tolerance Test.

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