RESEARCH PAPER

Evaluation of the Antidiabetic potential of quercetin-3-O-β-D-glucoside in insulin-resistant 3T3-L1 adipocytes cells through modulation of glucose uptake and AMPK signaling

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is characterized by chronic hyperglycemia, insulin resistance, oxidative stress, and inflammatory dysregulation. Adipose tissue dysfunction plays a major role in the progression of insulin resistance by impairing glucose uptake and metabolic homeostasis. The present study investigated the antidiabetic potential of quercetin-3-O-β-D-glucoside (isoquercitrin) in high glucose-induced insulin-resistant 3T3-L1 adipocytes, with emphasis on glucose uptake and AMPK-associated signaling pathways. Insulin resistance was induced in differentiated 3T3-L1 adipocytes under high glucose conditions, followed by treatment with isoquercitrin and metformin as the positive control. The effects of isoquercitrin were evaluated through assessment of glycogen content, antioxidant biomarkers (GPx and CAT), inflammatory mediators (NF-κB, TNF-α, and IL-6), and mRNA expression of GLUT-4, PPAR-γ, AMPK, and adiponectin. High glucose exposure significantly reduced glycogen accumulation, antioxidant enzyme levels, and expression of GLUT-4, PPAR-γ, AMPK, and adiponectin, while markedly increasing NF-κB, TNF-α, and IL-6 levels. Treatment with isoquercitrin significantly reversed these alterations in a dose-dependent manner, with the 50 μM treatment demonstrating effects comparable to metformin. Isoquercitrin restored antioxidant defense systems, suppressed inflammatory signaling, enhanced glycogen synthesis, and improved insulin-sensitive gene expression associated with glucose uptake and metabolic regulation. The findings suggest that quercetin-3-O-β-D-glucoside ameliorates insulin resistance in adipocytes through modulation of oxidative stress, inflammatory pathways, and AMPK-associated metabolic signaling. Therefore, isoquercitrin may serve as a promising natural therapeutic candidate for the management of insulin resistance and type 2 diabetes mellitus.

Keywords: Quercetin-3-O-β-D-glucoside, Type 2 diabetes mellitus, AMPK signaling, GLUT4 translocation, Antidiabetic activity.

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Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic metabolic disorders worldwide and is characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. The global increase in obesity, sedentary lifestyle, and unhealthy dietary habits has contributed significantly to the rising incidence of T2DM and its associated complications, including cardiovascular disease, nephropathy, neuropathy, and impaired wound healing (International Diabetes Federation, 2021). Among the major insulin-responsive tissues, adipose tissue plays a central role in maintaining systemic glucose and lipid homeostasis. In healthy adipocytes, insulin stimulates glucose uptake through translocation of glucose transporter-4 (GLUT4) to the plasma membrane, promotes glycogen synthesis, and regulates lipid storage. However, under diabetic and hyperglycemic conditions, adipocytes develop insulin resistance characterized by impaired glucose uptake, excessive lipid accumulation, oxidative stress, mitochondrial dysfunction, and chronic low-grade inflammation (Saltiel & Kahn, 2001). These alterations contribute substantially to the progression of metabolic syndrome and T2DM. Insulin-resistant adipocytes also exhibit elevated production of reactive oxygen species (ROS) and inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nuclear factor-kappa B (NF-κB), which interfere with insulin receptor signaling and exacerbate metabolic dysfunction (Hotamisligil, 2006).

Oxidative stress-mediated activation of inflammatory pathways further suppresses insulin sensitivity and impairs adipokine secretion, including adiponectin, an important insulin-sensitizing adipokine involved in glucose metabolism and fatty acid oxidation (Kadowaki & Yamauchi, 2005). Therefore, modulation of oxidative stress and inflammatory signaling pathways has emerged as an important therapeutic strategy for the management of insulin resistance and T2DM. AMP-activated protein kinase (AMPK) is a highly conserved cellular energy sensor that plays a crucial role in maintaining metabolic homeostasis by regulating glucose uptake, fatty acid oxidation, glycogen synthesis, and mitochondrial metabolism. Activation of AMPK enhances insulin sensitivity by promoting GLUT4 translocation, suppressing hepatic gluconeogenesis, and improving cellular energy balance (Hardie et al., 2012). Consequently, AMPK has become an important molecular target for antidiabetic therapies, including metformin, which exerts part of its glucose-lowering effect through AMPK activation (Foretz et al., 2022). Several phytochemicals and flavonoids have also been reported to improve insulin sensitivity through AMPK-associated pathways.

Natural flavonoids have attracted considerable interest as potential therapeutic agents for metabolic disorders due to their antioxidant, anti-inflammatory, and insulin-sensitizing properties. Among these compounds, isoquercitrin (quercetin-3-O-β-D-glucoside), a glycosylated derivative of quercetin, has emerged as a promising bioactive flavonoid with significant pharmacological activities. Isoquercitrin is widely distributed in fruits, vegetables, medicinal plants, and dietary herbs and has been reported to possess antioxidant, anti-inflammatory, anti-obesity, and antidiabetic properties (Li et al., 2016). Compared with quercetin aglycone, isoquercitrin demonstrates improved water solubility and bioavailability, making it more effective in modulating metabolic pathways associated with diabetes and insulin resistance. Previous investigations have demonstrated that quercetin and its glycosides exert beneficial effects in several insulin-sensitive tissues, including adipose tissue, skeletal muscle, hepatocytes, and pancreatic β-cells (Eid & Haddad, 2017). The antidiabetic effects of isoquercitrin are strongly associated with enhanced glucose uptake, GLUT4 translocation, antioxidant defense, and modulation of AMPK-related signaling pathways. Kobori et al. (2020) reported that quercetin glycosides promoted GLUT4 translocation and improved hyperglycemia through activation of the Ca2+/calmodulin-dependent protein kinase kinase-β (CaMKKβ)/AMPK signaling pathway without directly stimulating insulin receptor signaling. Similarly, Eid et al. (2015) demonstrated that isoquercitrin stimulated glucose uptake in rat skeletal muscle through mechanisms involving phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and GLUT4 translocation pathways. In hepatocyte models, Lee et al. (2019) observed that quercetin and isoquercitrin suppressed hepatic gluconeogenesis by increasing liver kinase B1 (LKB1) expression and AMPKα phosphorylation, producing metabolic effects comparable to metformin.

Although direct evidence regarding isoquercitrin activity in insulin-resistant adipocytes remains limited, several studies support the beneficial effects of quercetin-derived compounds in adipose tissue metabolism. Arias et al. (2018) reported that quercetin metabolites reduced lipid accumulation and improved glucose metabolism in high glucose-treated 3T3-L1 adipocytes, potentially through AMPK activation and regulation of adipogenic signaling pathways. Likewise, Ahn et al. (2008) demonstrated that quercetin inhibited adipogenesis and lipid accumulation in mature 3T3-L1 adipocytes through modulation of adipocyte differentiation-related genes. More recently, Zhang et al. (2021) reported that isoquercitrin improved glucose uptake and attenuated oxidative stress in insulin-resistant hepatocyte models through activation of antioxidant and AMPK-associated pathways. Collectively, these findings suggest that isoquercitrin may improve insulin sensitivity and restore metabolic flexibility in adipocytes through enhancement of glucose uptake and modulation of AMPK-mediated signaling pathways. The 3T3-L1 adipocyte model is a well-established in vitro system widely used for investigating adipogenesis, glucose metabolism, insulin resistance, and antidiabetic drug activity. Exposure of differentiated 3T3-L1 adipocytes to high glucose conditions induces insulin resistance-associated metabolic alterations, including impaired glucose uptake, oxidative stress, inflammatory activation, and dysregulated adipokine expression, thereby closely mimicking diabetic adipocyte dysfunction (Green & Kehinde, 1975). Therefore, this model provides an effective platform for evaluating the therapeutic potential of natural bioactive compounds against insulin resistance. Based on these considerations, the present study aimed to investigate the antidiabetic potential of quercetin-3-O-β-D-glucoside in high glucose-induced insulin-resistant 3T3-L1 adipocytes. Particular emphasis was placed on evaluating its effects on glucose uptake, glycogen accumulation, antioxidant defense systems, inflammatory mediators, adipokine regulation, and AMPK-associated insulin-sensitive signaling pathways. The findings of this study may contribute to the development of isoquercitrin as a promising natural

therapeutic candidate for the management of insulin resistance and type 2 diabetes mellitus.

Materials and Methods

Chemicals and reagents

Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), penicillin/streptomycin antibiotics, Propidium Iodide (PI) stain, Dimethyl sulfoxide (DMSO), RNase, metabolic assay kits (Glucose uptaken and Lactose production) were purchased from HiMedia and abcam. Quercetin was purchased from Sigma Aldrich, India. The total RNA isolation kit was provided by Invitrogen, USA. The primers were provided by Eurofins Genomics India Pvt. Ltd, Bangalore, India.

Cell lines

A 3T3-L1 cell line was procured from the American Type Culture Collection (ATCC), Manassas, Virginia, USA. The cells were maintained and grown in a controlled environment inside a CO_2 incubator set at a temperature of 37°C. DMEM, together with 10% FBS and 1% penicillin-streptomycin antibiotics, was the culture media utilised for cell growth.

Cell Viability

The cytotoxic potential of qurcetin-3-O-β-D-glucoside against diabetic cells was evaluated using the MTT colorimetric assay. Briefly, cells were seeded at a density of 1×10^4 cells per well in 96-well plates and allowed to adhere overnight. Qurcetin-3-O-β-D-glucoside was prepared at different concentrations in serum-free DMEM and added to the respective wells. Following 24 hours of incubation, the treatment medium was discarded, and 100 µL of MTT solution was added to each well and incubated for 1 hour. Subsequently, the formazan crystals formed were dissolved using DMSO, and absorbance was measured at 590 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells.

Glycogen Estimation

Cellular glycogen content was determined using the anthrone method. Briefly, treated 3T3-L1 adipocytes were lysed, and glycogen was extracted using alkaline digestion. The extract was reacted with freshly prepared anthrone reagent in concentrated sulfuric acid and heated in a boiling water bath. After cooling, the absorbance was measured spectrophotometrically at 620 nm. Glycogen concentration was calculated using a glucose standard curve and expressed as mg/g of sample.

Estimation of GPx, CAT, NF-κB, and IL-6 by ELISA

The levels of glutathione peroxidase (GPx), catalase (CAT), nuclear factor-kappa B (NF-κB), and interleukin-6 (IL-6) in treated 3T3-L1 adipocytes were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Briefly, cell lysates collected from control and treated groups were added to antibody-coated 96-well microplates and incubated under specified conditions. After washing to remove unbound substances, enzyme-conjugated secondary antibodies were added followed by incubation with chromogenic substrate solution. The reaction was terminated using stop solution, and absorbance was measured at 450 nm using a microplate reader. The concentrations of GPx, CAT, NF-κB, and IL-6 were calculated from their respective standard calibration curves and expressed as ng/mL or pg/mL according to the assay specifications.

Gene expression analysis

RNA Isolation and cDNA Synthesis

Total RNA was isolated from 3T3-L1 cell pellets using TRIR reagent (Invitrogen) according to the manufacturer's instructions. Briefly, the harvested cell pellet was lysed in 1 mL of TRIR reagent, followed by the addition of chloroform and centrifugation at 12,000 ×g for 15 min at 4°C. The resulting aqueous phase was carefully collected, and RNA was precipitated with isopropanol, washed with 75% ethanol, and finally dissolved in RNase-free water. The concentration and purity of the isolated RNA were assessed spectrophotometrically. Subsequently, 2 µg of total RNA was used for complementary DNA (cDNA) synthesis using a reverse transcription kit according to the manufacturer's protocol.

Quantitative Real-Time PCR (qRT-PCR)

Gene expression analysis was performed using SYBR Green-based real-time PCR (Takara). The reaction mixture consisted of 2× SYBR Green master mix, gene-specific forward and reverse primers, and nuclease-free water. Amplification was carried out using a Stratagene MX3000P system (Agilent Technologies) under the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 20 s, and extension at 72°C for 40 s. The mRNA expression levels of pro-apoptotic markers were quantified. β-actin was used as the internal control. Relative gene expression was calculated using comparative Ct (ΔΔCt) method based on amplification and melt curve analysis.

Statistical Analysis

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the control and treatment groups using a computer - based software. In Duncan's multiple range test, the significance was considered at the level of $p<0.05$.

Results

Cytotoxicity

The cytoprotective effect of quercetin-3-O-β-D-glucoside against high glucose-induced cellular damage in 3T3-L1 adipocytes was evaluated using the MTT assay. The control group exhibited 100% cell viability, indicating normal metabolic activity and cellular integrity. In contrast, the high glucose (HG) group showed a marked reduction in cell viability to 65%, confirming that hyperglycemic conditions induced significant cellular stress and cytotoxicity in adipocytes. Treatment with quercetin-3-O-β-D-glucoside significantly improved cell viability in a dose-dependent manner. The HG+25 µM group demonstrated increased viability of 79%, while the HG+50 µM group showed a further enhancement to 90%, indicating substantial protection against glucose-induced cellular injury. The metformin-treated group exhibited 95% viability, which was comparable to the higher concentration of quercetin-3-O-β-D-glucoside. These findings suggest that quercetin-3-O-β-D-glucoside effectively protects insulin-resistant adipocytes from high glucose-mediated cytotoxicity and may improve cellular metabolic activity through its antioxidant and insulin-sensitizing properties. The observed increase in viability further supports the therapeutic potential of quercetin-3-O-β-D-glucoside in maintaining adipocyte function under diabetic conditions.

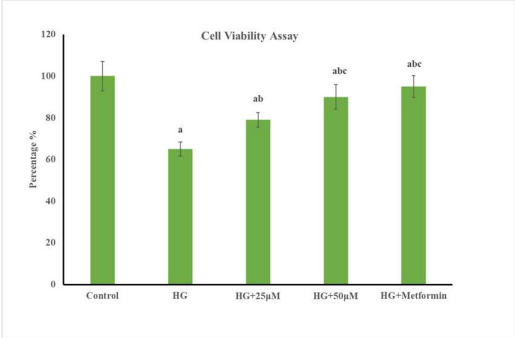


Figure 1: Effect of quercetin-3-O-β-D-glucoside on cell viability in high glucose-induced insulin-resistant 3T3-L1 adipocytes. High glucose exposure significantly reduced adipocyte viability compared to the control group, whereas treatment with quercetin-3-O-β-D-glucoside (25 and 50 µM) dose-dependently restored cell viability. Metformin-treated cells showed near-normal viability. Data are expressed as mean ± SEM. Statistical significance was considered at $p < 0.05$.

GLUT-4 mRNA Expression

The HG-treated group showed a marked reduction in GLUT-4 mRNA expression compared with the control group, indicating impaired glucose transport and insulin resistance in 3T3-L1 adipocytes. Treatment with quercetin-3-O-β-D-glucoside significantly increased GLUT-4 expression in a dose-dependent manner. The 25 µM treatment partially restored GLUT-4 levels, whereas the 50 µM treatment showed expression levels close to the metformin-treated group. These findings suggest that quercetin-3-O-β-D-glucoside improves insulin sensitivity and enhances glucose uptake by stimulating GLUT-4 gene expression.

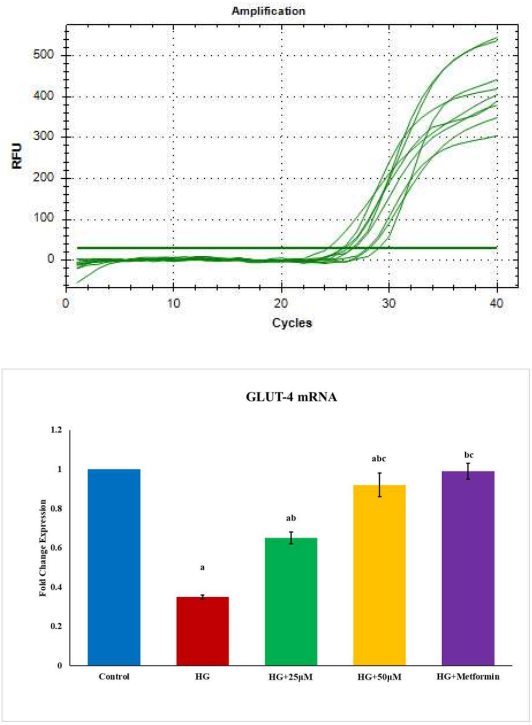


Figure 2 Effect of quercetin-3-O-β-D-glucoside on GLUT-4 mRNA expression in insulin-resistant 3T3-L1 adipocytes. High-glucose (HG) treatment significantly reduced GLUT-4 expression compared with the control group, whereas treatment with quercetin-3-O-β-D-glucoside (25 and 50 µM) significantly restored GLUT-4 mRNA levels in a dose-dependent manner. Metformin served as the positive control. Data are expressed as mean ± SEM. Different superscript letters indicate statistically significant differences at $p < 0.05$.

PPAR-γ mRNA Expression

PPAR-γ mRNA expression was significantly decreased in HG-induced insulin-resistant adipocytes compared with normal control cells, confirming disrupted adipocyte differentiation and glucose metabolism under hyperglycemic conditions. Treatment with quercetin-3-O-β-D-glucoside significantly elevated PPAR-γ expression in a concentration-dependent manner. The 50 µM-treated group demonstrated expression levels comparable to metformin, indicating improved insulin responsiveness and regulation of lipid and glucose metabolism through activation of PPAR-γ signaling pathways.

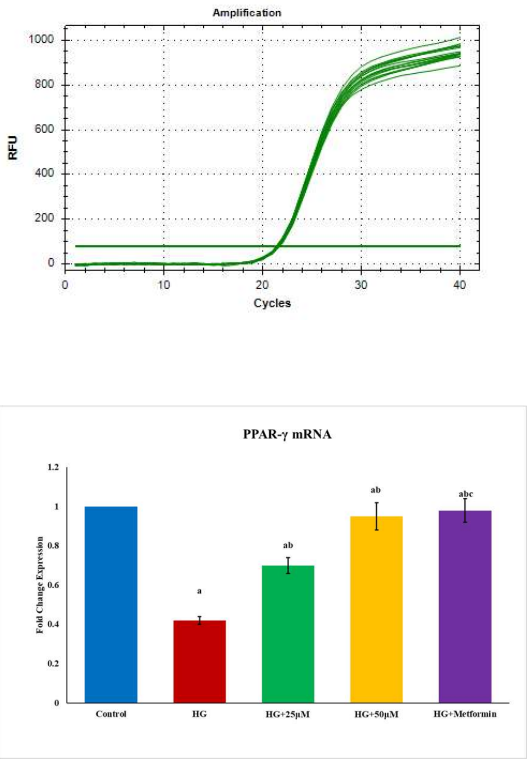


Figure 3 Effect of quercetin-3-O-β-D-glucoside on PPAR-γ mRNA expression in insulin-resistant 3T3-L1 adipocytes. HG exposure significantly downregulated PPAR-γ expression, while treatment with quercetin-3-O-β-D-glucoside increased expression levels dose-dependently. The 50 μM treatment showed effects comparable to metformin. Data are expressed as mean ± SEM. Different superscript letters indicate statistically significant differences at $p < 0.05$.

AMPK mRNA Expression

HG exposure significantly suppressed AMPK mRNA expression compared with the control group, reflecting impaired cellular energy metabolism and reduced insulin signaling activity. Administration of quercetin-3-O-β-D-glucoside significantly upregulated AMPK expression in treated cells. The increase was more pronounced at 50 μM and was comparable to the metformin-treated group. These findings indicate that quercetin-3-O-β-D-glucoside activates AMPK signaling, which may contribute to enhanced glucose uptake, improved metabolic homeostasis, and reduced insulin resistance.

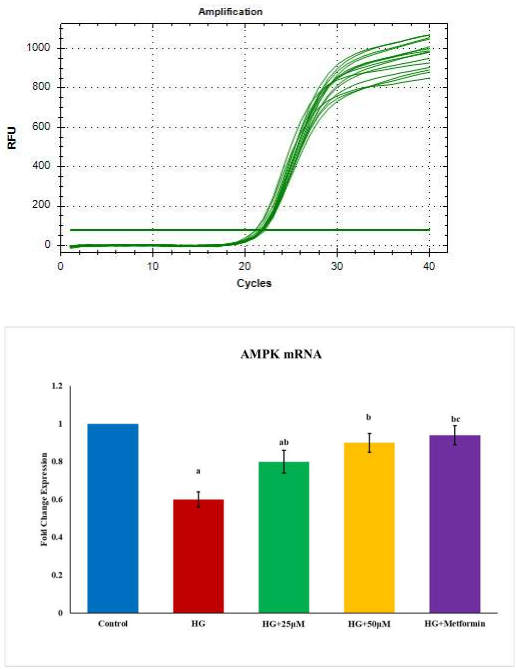


Figure 4 Effect of quercetin-3-O-β-D-glucoside on AMPK mRNA expression in insulin-resistant 3T3-L1 adipocytes. HG treatment significantly suppressed AMPK expression compared with control cells. Treatment with quercetin-3-O-β-D-glucoside significantly increased AMPK mRNA expression, indicating activation of metabolic signaling pathways associated with glucose homeostasis. Data are expressed as mean ± SEM. Different superscript letters indicate statistically significant differences at $p < 0.05$.

Adiponectin mRNA Expression

Adiponectin mRNA expression was markedly reduced in the HG group, indicating impaired adipocyte function and decreased insulin sensitivity under hyperglycemic conditions. Treatment with quercetin-3-O-β-D-glucoside restored adiponectin expression in a dose-dependent manner. The 50 μM treatment produced a substantial increase in adiponectin levels, closely resembling the metformin-treated group. This restoration suggests that quercetin-3-O-β-D-glucoside improves adipocyte metabolic function and enhances insulin sensitivity through modulation of adiponectin-associated signaling pathways.

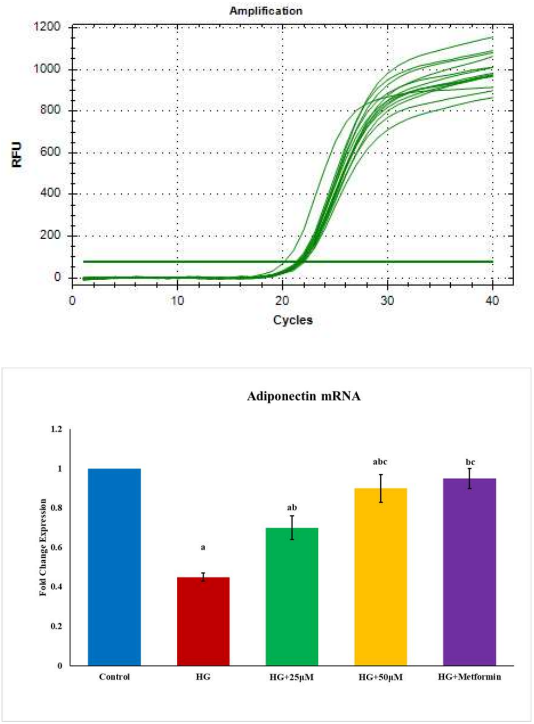


Figure 5 Effect of quercetin-3-O-β-D-glucoside on adiponectin mRNA expression in insulin-resistant 3T3-L1 adipocytes. HG-induced insulin resistance significantly reduced adiponectin expression, whereas quercetin-3-O-β-D-glucoside treatment restored adiponectin levels in a concentration-dependent manner. Metformin-treated cells showed similar effects. Data are expressed as mean ± SEM. Different superscript letters indicate statistically significant differences at $p < 0.05$.

TNF-α mRNA Expression

The HG-treated group exhibited a significant increase in TNF-α mRNA expression compared with the control group, indicating enhanced inflammatory responses and the establishment of insulin-resistant conditions in 3T3-L1 adipocytes. Treatment with quercetin-3-O-β-D-glucoside significantly reduced TNF-α expression in a dose-dependent manner. The 25 µM treatment partially suppressed TNF-α levels, while the 50 µM treatment demonstrated a greater reduction, approaching the effect observed in the metformin-treated group. These findings suggest that quercetin-3-O-β-D-glucoside effectively attenuates hyperglycemia-induced inflammation by downregulating pro-inflammatory cytokine expression, thereby contributing to improved insulin sensitivity and metabolic function.

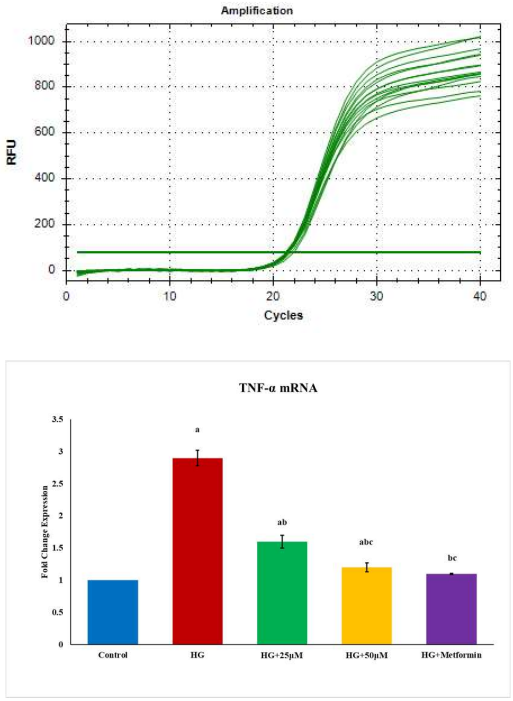


Figure 6 Effect of quercetin-3-O-β-D-glucoside on TNF-α mRNA expression in insulin-resistant 3T3-L1 adipocytes. High-glucose (HG) treatment significantly increased TNF-α expression compared with the control group, indicating activation of inflammatory signaling pathways. Treatment with quercetin-3-O-β-D-glucoside (25 and 50 µM) significantly reduced TNF-α mRNA expression in a dose-dependent manner, with effects comparable to metformin at higher concentration. Data are expressed as mean ± SEM. Different superscript letters indicate statistically significant differences at $p < 0.05$.

IL-6 mRNA Expression

The IL-6 mRNA expression analysis revealed a significant increase in the HG-treated group compared to the control, indicating that high glucose conditions induced a strong inflammatory response in insulin-resistant 3T3-L1 adipocytes. Treatment with quercetin-3-O-β-D-glucoside (Q3G) at 25 µM significantly reduced IL-6 expression when compared to the HG group, while the 50 µM concentration further suppressed IL-6 levels, demonstrating a dose-dependent anti-inflammatory effect. Metformin treatment also markedly decreased IL-6 expression and restored the levels close to the control group. These findings suggest that Q3G effectively attenuates glucose-induced inflammation by regulating pro-inflammatory cytokine expression in adipocyte cells.

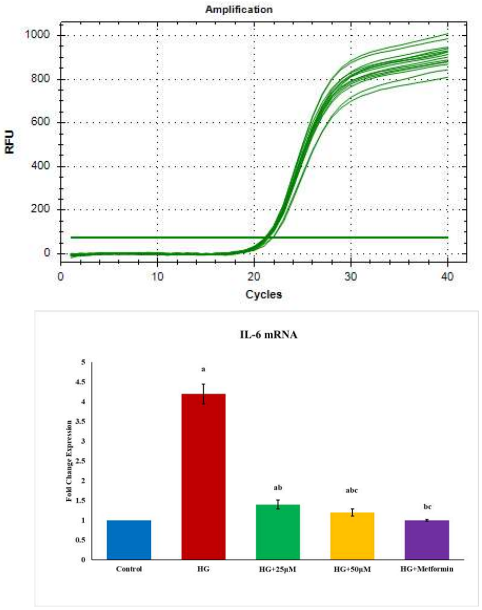
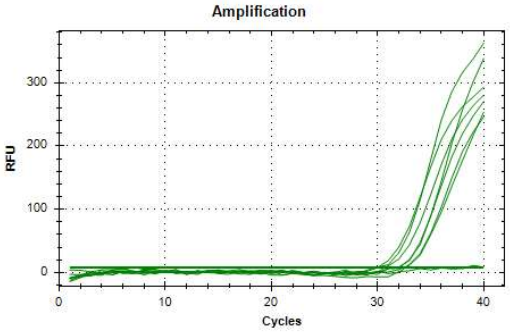


Figure 7 Effect of quercetin-3-O-β-D-glucoside (Q3G) on IL-6 mRNA expression in insulin-resistant 3T3-L1 adipocytes. HG exposure significantly increased IL-6 expression, while Q3G and metformin treatments reduced inflammatory gene expression in a dose-dependent manner.

NF-κB mRNA Expression

NF-κB mRNA expression was markedly elevated in the HG-treated cells compared to the control group, confirming activation of inflammatory signaling pathways under hyperglycemic conditions. Administration of Q3G significantly decreased NF-κB expression at both 25 µM and 50 µM concentrations, with the higher dose exhibiting stronger inhibitory activity. Metformin treatment also reduced NF-κB expression to near-normal levels. The suppression of NF-κB expression by Q3G indicates its protective role against glucose-induced inflammatory stress and suggests its potential involvement in modulating inflammatory and insulin-resistance-associated signaling pathways.



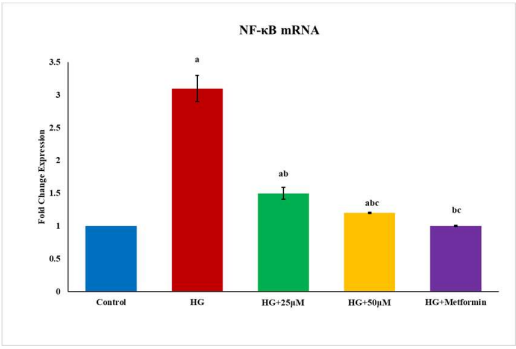


Figure 8 Effect of quercetin-3-O-β-D-glucoside (Q3G) on NF-κB mRNA expression in insulin-resistant 3T3-L1 adipocytes. Q3G treatment markedly suppressed HG-induced NF-κB expression, demonstrating its potential anti-inflammatory activity comparable to metformin.

Effect of quercetin-3-O-β-D-glucoside on glycogen content, antioxidant enzymes, and inflammatory biomarkers

High-glucose (HG) exposure significantly altered glucose metabolism, oxidative stress markers, and inflammatory mediators in 3T3-L1 adipocytes compared with the control group. Glycogen content was markedly reduced in the HG-treated cells, indicating impaired glucose utilization and insulin resistance. Treatment with quercetin-3-O-β-D-glucoside significantly restored glycogen levels in a dose-dependent manner, with the 50 µM treatment showing greater improvement and values approaching those observed in the metformin-treated group. This suggests enhanced glucose uptake and glycogen synthesis following treatment. The antioxidant biomarkers GPx and CAT were significantly decreased in the HG group, reflecting elevated oxidative stress under hyperglycemic conditions. Treatment with quercetin-3-O-β-D-glucoside significantly increased both GPx and CAT levels compared with the HG group. The restoration was more pronounced at 50 µM concentration, indicating potent antioxidant activity and protection against oxidative damage in insulin-resistant adipocytes. Inflammatory biomarkers NF-κB and IL-6 were markedly elevated in the HG group, confirming activation of inflammatory signaling pathways associated with insulin resistance. Administration of quercetin-3-O-β-D-glucoside significantly reduced NF-κB and IL-6 levels in a concentration-dependent manner. The 50 µM-treated group demonstrated inflammatory marker levels close to those of the metformin-treated group, suggesting strong anti-inflammatory effects of the compound. Overall, these findings demonstrate that quercetin-3-O-β-D-glucoside ameliorates hyperglycemia-induced metabolic dysfunction, oxidative stress, and inflammation in insulin-resistant adipocytes.

Biomarkers	Control	HG	HG+ 25µM	HG+ 50µM	HG+ Metformin
Glycogen (mg/g)	16± 1.1	7±0 .4[a]	10±0. 9[ab]	12±0. 4[ab]	17±1. 1[bcd]
GPx (ng/mL)	49± 1.8	20± 1.5[a]	30±1. 5[ab]	36±1. 8[abc]	49±2. 5[bcd]
CAT (Pg/mL)	50± 3.5	16± 1.1[a]	29±1. 7[ab]	39±1. 8[abc]	45±2. 5[bcd]
NF-κB (ng/mL)	22± 1.1	49± 1.8[a]	35±1. 8[ab]	30±1. 6[abc]	28±1. 7[bcd]
IL-6 (ng/mL)	19± 1.1	70± 1.5[a]	40±1. 8[ab]	25±1. 8[abc]	17±1. 1[bcd]

Table 1 Effect of quercetin-3-O-β-D-glucoside on glycogen content, antioxidant enzymes (GPx and CAT), and inflammatory biomarkers (NF-κB and IL-6) in insulin-resistant 3T3-L1 adipocytes. High-glucose (HG) treatment significantly impaired glycogen metabolism, reduced antioxidant enzyme levels, and increased inflammatory mediators compared with control cells. Treatment with quercetin-3-O-β-D-glucoside (25 and 50 µM) significantly restored glycogen, GPx, and CAT levels while reducing NF-κB and IL-6 expression in a dose-dependent manner. Metformin was used as the positive control. Data are expressed as mean ± SEM. Different superscript letters indicate statistically significant differences at $p < 0.05$.

Discussion

The present study demonstrates that quercetin-3-O-β-D-glucoside (isoquercitrin) exerts significant antidiabetic effects in high glucose-induced insulin-resistant 3T3-L1 adipocytes through modulation of glucose metabolism, oxidative stress, inflammatory mediators, and AMPK-associated signaling pathways. Exposure to high glucose markedly suppressed the expression of GLUT-4, PPAR-γ, AMPK, and adiponectin, reduced glycogen accumulation and antioxidant enzyme levels, and simultaneously increased inflammatory cytokines including TNF-α, IL-6, and NF-κB. Treatment with isoquercitrin significantly reversed these alterations in a dose-dependent manner, with the 50 µM-treated group exhibiting effects comparable to metformin. These findings suggest that isoquercitrin improves adipocyte insulin responsiveness and metabolic homeostasis under hyperglycemic stress conditions. Insulin resistance is primarily characterized by defective glucose uptake and impaired insulin signaling in peripheral tissues such as adipose tissue and skeletal muscle. GLUT-4 is the principal insulin-responsive glucose transporter responsible for glucose uptake in adipocytes, and reduced GLUT-4 expression is strongly associated with insulin resistance and hyperglycemia. In the present study, HG exposure markedly decreased GLUT-4 mRNA expression, whereas isoquercitrin treatment restored its expression in a concentration-dependent manner. Similar observations were reported by Yamashita et al. (2024), who demonstrated that quercetin glycosides promoted GLUT-4 translocation in skeletal muscle through activation of the CaMKKβ/AMPK pathway, thereby improving glucose uptake and preventing hyperglycemia. Likewise, Kobori et al. (2020) showed that long-term administration of quercetin glycosides in high-fat diet-fed mice increased AMPK phosphorylation and GLUT-4 translocation while reducing plasma glucose and insulin resistance. These findings collectively support the possibility that isoquercitrin improves glucose handling through AMPK-associated regulation of GLUT-4 signaling.

PPAR-γ is a key transcription factor regulating adipocyte differentiation, lipid metabolism, and insulin sensitivity. Suppression of PPAR-γ contributes to adipocyte dysfunction and impaired metabolic regulation under diabetic conditions. In the current study, isoquercitrin restored PPAR-γ expression in HG-treated adipocytes, indicating improved adipogenic and metabolic signaling. Similar findings were reported by Eid et al. (2018), who demonstrated that flavonoids including quercetin derivatives modulate PPAR-γ signaling pathways and improve insulin sensitivity by regulating genes associated with glucose and lipid metabolism. Restoration of PPAR-γ may therefore contribute to the improved glycogen synthesis and metabolic recovery observed in the treated groups. AMPK functions as a central cellular energy sensor regulating glucose uptake, lipid oxidation, and insulin signaling. Hyperglycemic stress significantly reduced AMPK expression in insulin-resistant adipocytes, whereas isoquercitrin restored AMPK mRNA expression in a dose-dependent manner. These findings are consistent with reports by Kobori et al. (2020), who observed increased AMPK phosphorylation in adipose tissue, liver, and skeletal muscle following quercetin glycoside administration in obese mice. Similarly, Yamashita et al. (2024) reported that quercetin glycosides enhanced GLUT-4 translocation through AMPK activation independent of classical insulin signaling pathways. In HepG2 hepatocytes, Zhang et al. (2023) demonstrated that isoquercitrin activated AMPK signaling and suppressed SREBP-1 and fatty acid synthase (FAS)-mediated lipogenesis, thereby improving metabolic homeostasis. Moreover, quercetin metabolites were reported to increase phosphorylated AMPK levels and reduce lipid accumulation under high-glucose conditions.

However, the mechanistic interpretation of the present findings should be approached cautiously. Although AMPK mRNA expression was elevated following isoquercitrin treatment, AMPK activation is primarily dependent on phosphorylation at Thr172 rather than transcriptional upregulation alone. As highlighted by Foretz et al. (2022), AMPK-mediated glucose uptake and GLUT-4 translocation are more accurately associated with phosphorylated AMPK (p-AMPK) levels than with AMPK gene expression alone. Therefore, the present findings support AMPK-associated metabolic modulation rather than conclusively establishing direct pathway activation. Adiponectin is an insulin-sensitizing adipokine that enhances fatty acid oxidation and glucose uptake through AMPK-mediated pathways. Reduced adiponectin levels are closely associated with obesity, insulin resistance, and chronic inflammation. In the present study, adiponectin expression was markedly suppressed under HG conditions but restored following isoquercitrin treatment. Similar effects were reported by Rivera et al. (2024), who observed improved adiponectin signaling and insulin responsiveness following quercetin supplementation in metabolic disorder models. Elevated adiponectin expression may contribute to downstream activation of AMPK signaling and restoration of adipocyte metabolic function.

Oxidative stress is a major contributor to insulin resistance and diabetic complications. Chronic hyperglycemia promotes excessive reactive oxygen species (ROS) production, resulting in mitochondrial dysfunction and impaired insulin signaling. In the present study, HG exposure significantly reduced GPx and CAT levels, confirming oxidative stress-mediated cellular injury. Isoquercitrin treatment restored antioxidant enzyme levels, indicating potent antioxidant activity. According to Boots et al. (2008) and Li et al. (2014), quercetin glycosides exhibit strong antioxidant and cytoprotective effects through free radical scavenging, ROS neutralization, and modulation of antioxidant defense pathways including Nrf2 and AMPK signaling. Restoration of antioxidant defenses may therefore protect adipocytes from oxidative injury and improve insulin sensitivity. Inflammation is another critical factor contributing to insulin resistance and metabolic dysfunction. Hyperglycemia-induced oxidative stress activates NF-κB signaling pathways, which subsequently stimulate the release of pro-inflammatory cytokines such as TNF-α and IL-6. These cytokines impair insulin receptor signaling and worsen metabolic imbalance. In the present study, HG exposure significantly elevated NF-κB, TNF-α, and IL-6 expression, whereas isoquercitrin treatment markedly suppressed these inflammatory mediators. Similar anti-inflammatory effects were reported by Rivera et al. (2024) and Zhang et al. (2025), who demonstrated that quercetin derivatives regulate inflammatory signaling pathways including NF-κB while simultaneously improving glucose metabolism and insulin sensitivity. Therefore, suppression of inflammatory signaling likely contributes substantially to the insulin-sensitizing effects observed in the current study. The restoration of glycogen levels in isoquercitrin-treated cells further supports improved glucose metabolism and intracellular glucose utilization. Glycogen synthesis is a downstream consequence of effective insulin signaling and glucose uptake. The increase in glycogen accumulation observed in treated groups is therefore consistent with enhanced GLUT-4 expression, improved AMPK-associated signaling, and restoration of adipocyte metabolic function.

Despite these promising findings, several limitations should be acknowledged. The present investigation was conducted exclusively in an in vitro 3T3-L1 adipocyte model, which does not fully replicate the complex interactions among adipose tissue, skeletal muscle, liver, pancreatic β-cells, and immune cells observed in vivo. Additionally, the study evaluated AMPK mRNA expression rather than phosphorylated AMPK protein levels, limiting definitive conclusions regarding pathway activation. Similarly, increased GLUT-4 transcription does not directly confirm membrane translocation or enhanced insulin-stimulated glucose uptake. Future studies should therefore include assessment of phosphorylated AMPK (p-AMPK), acetyl-CoA carboxylase phosphorylation (p-ACC), GLUT-4 membrane translocation assays, glucose uptake analysis, and AMPK inhibition or knockdown experiments to establish mechanistic causality. In vivo diabetic animal studies and pharmacokinetic investigations are also necessary to validate the therapeutic efficacy and bioavailability of isoquercitrin under physiological conditions. Overall, the present study demonstrates that quercetin-3-O-β-D-glucoside ameliorates insulin resistance in high glucose-induced 3T3-L1 adipocytes through enhancement of glucose metabolism, restoration of antioxidant defenses, suppression of inflammatory signaling, and modulation of AMPK-associated metabolic pathways. These findings suggest that isoquercitrin may serve as a promising natural therapeutic candidate for the management of insulin resistance and type 2 diabetes mellitus.

Conclusion

The present study demonstrates that quercetin-3-O-β-D-glucoside (isoquercitrin) possesses significant antidiabetic activity in high glucose-induced insulin-resistant 3T3-L1 adipocytes. Isoquercitrin effectively restored glucose metabolism by increasing glycogen accumulation and enhancing the expression of insulin-sensitive genes including

GLUT-4, PPAR-γ, AMPK, and adiponectin. In addition, the compound markedly improved antioxidant defense mechanisms through elevation of GPx and CAT levels while suppressing inflammatory mediators such as NF-κB, TNF-α, and IL-6. These findings indicate that isoquercitrin ameliorates insulin resistance by modulating oxidative stress, inflammatory signaling, and AMPK-associated metabolic pathways. The 50 μM treatment exhibited effects comparable to metformin, highlighting the therapeutic potential of isoquercitrin as a natural antidiabetic agent. However, the present study is limited to an in vitro adipocyte model, and further investigations involving phosphorylated AMPK analysis, GLUT4 translocation studies, mechanistic pathway validation, and in vivo diabetic models are necessary to confirm its molecular mechanisms and clinical relevance. Overall, isoquercitrin represents a promising phytochemical candidate for the development of novel therapeutic strategies against insulin resistance and type 2 diabetes mellitus.

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