RESEARCH PAPER

Investigation of Europetin-3-O-Rhamnoside as a novel natural insulin-sensitizing compound in 3T3-L1 Adipocytes via regulation of GLUT-4 and adiponectin expression

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by impaired insulin signaling, reduced glucose uptake, oxidative stress, and adipocyte dysfunction. The present study investigated the antidiabetic potential of Europetin-3-O-rhamnoside as a novel natural insulin-sensitizing compound in high glucose-induced insulin-resistant 3T3-L1 adipocytes through modulation of GLUT4 and adiponectin-associated signaling pathways. Insulin resistance was induced in differentiated 3T3-L1 adipocytes using high-glucose conditions, followed by treatment with Europetin-3-O-rhamnoside at different concentrations. Cell viability was evaluated using the MTT assay, while gene expression analysis of insulin receptor (IR), insulin receptor substrate (IRS), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), and glucose transporter-4 (GLUT4) was performed using quantitative real-time PCR. Oxidative stress and antioxidant biomarkers including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and hydrogen peroxide (H_2O_2) were also assessed. High-glucose exposure significantly reduced IR, IRS, PI3K, AKT, and GLUT4 expression levels while increasing oxidative stress, confirming the establishment of insulin resistance in adipocytes. Treatment with Europetin-3-O-rhamnoside significantly restored insulin-signaling gene expression in a dose-dependent manner, with the 20 µg/mL group exhibiting effects comparable to metformin-treated cells. Furthermore, Europetin-3-O-rhamnoside enhanced antioxidant defense systems by increasing SOD, CAT, and GSH levels while reducing H_2O_2 accumulation. The findings suggest that Europetin-3-O-rhamnoside ameliorates insulin resistance through restoration of the PI3K/AKT/GLUT4 signaling pathway and attenuation of oxidative stress-mediated cellular dysfunction. Overall, the study demonstrates that Europetin-3-O-rhamnoside possesses promising insulin-sensitizing and antioxidant properties and may serve as a potential natural therapeutic candidate for the management of insulin resistance and type 2 diabetes mellitus.

Keywords: Europetin-3-O-rhamnoside; Type 2 diabetes mellitus; Insulin resistance; 3T3-L1 adipocytes; GLUT4; PI3K/AKT signaling; Oxidative stress; Antioxidant activity.

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, defective insulin signaling, and peripheral insulin resistance. Among insulin-responsive tissues, adipose tissue plays a pivotal role in maintaining systemic glucose and lipid homeostasis through regulation of glucose uptake, fatty acid metabolism, adipokine secretion, and energy balance. In recent years, insulin resistance in adipocytes has no longer been considered merely a defect in glucose uptake but rather a complex disruption of the insulin-signaling network involving insulin receptor (IR), insulin receptor substrate (IRS), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), and glucose transporter-4 (GLUT4)-mediated pathways. Current mechanistic reviews identify the AKT-AS160-GLUT4 signaling axis as a central regulator of glucose homeostasis, where impaired GLUT4 translocation in adipose and skeletal muscle tissues contributes significantly to insulin resistance and metabolic dysfunction (Klip et al., 2019). Under physiological conditions, insulin binding to the insulin receptor activates IRS proteins and PI3K signaling, leading to phosphorylation of AKT and subsequent translocation of GLUT4-containing vesicles to the plasma membrane for glucose uptake. However, chronic hyperglycemia

and excessive nutrient exposure disrupt these signaling pathways, resulting in reduced insulin sensitivity and impaired glucose transport. Modern concepts of insulin resistance describe it as a multifactorial metabolic state associated with lipotoxicity, excessive adiposity, mitochondrial dysfunction, elevated free fatty acids, endoplasmic reticulum stress, oxidative stress, inflammatory signaling, and dysregulated adipokine secretion (Petersen & Shulman, 2018). These pathological alterations collectively impair insulin-mediated glucose metabolism and contribute to the progression of T2DM and related metabolic complications.

Oxidative stress has emerged as a major pathogenic factor linking obesity, chronic inflammation, and insulin resistance. Excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) under hyperglycemic conditions disrupts insulin signaling pathways by inducing oxidative damage to proteins, lipids, and mitochondria. According to Furukawa et al. (2017), oxidative stress in adipocytes activates stress-sensitive kinases and inflammatory mediators that impair insulin signaling and GLUT4 translocation. Recent reviews further emphasize that obesity-associated oxidative stress contributes directly to chronic low-grade inflammation and insulin resistance through activation of nuclear factor-kappa B (NF-κB), c-Jun N-terminal kinase (JNK), and inflammatory cytokines such as TNF-α and IL-6 (Manna & Jain, 2022). Similarly, a 2024 review by Zhao et al. highlighted oxidative stress, inflammation, adiposity, and mitochondrial dysfunction as major interconnected contributors to insulin resistance and metabolic syndrome. Therefore, modulation of oxidative stress and inflammatory pathways has become an important therapeutic strategy for improving insulin sensitivity. Adipokines secreted by adipose tissue also play critical roles in regulating insulin responsiveness and metabolic homeostasis. Among these, adiponectin is an insulin-sensitizing adipokine that enhances fatty acid oxidation and glucose uptake through activation of AMP-activated protein kinase (AMPK) and AKT signaling pathways. Reduced adiponectin expression is strongly associated with obesity, insulin resistance, and chronic inflammation (Kadowaki & Yamauchi, 2005). AMPK functions as a cellular energy sensor regulating glucose uptake, glycogen synthesis, lipid oxidation, and mitochondrial metabolism. Activation of AMPK improves insulin sensitivity by stimulating GLUT4 translocation and suppressing gluconeogenic and inflammatory signaling pathways (Hardie et al., 2012). Consequently, AMPK-associated signaling pathways are considered important molecular targets in the development of antidiabetic therapies.

Natural flavonoids have attracted considerable attention as potential insulin-sensitizing agents because of their antioxidant, anti-inflammatory, and metabolic regulatory activities. Among these phytochemicals, quercetin and its glycosylated derivatives have been extensively investigated for their beneficial effects on glucose metabolism and insulin signaling. A recent review by Rivera et al. (2024) concluded that quercetin and its metabolites improve insulin resistance through reduction of oxidative stress, suppression of inflammatory pathways, and enhancement of insulin-sensitive signaling mechanisms. Earlier reviews also demonstrated that flavonoids can regulate GLUT4 expression, improve AKT signaling, and modulate adipocyte metabolism, although detailed molecular studies in humans remain limited (Hanhineva et al., 2010). Experimental evidence further supports the antidiabetic potential of flavonoid compounds in adipocyte models. Ahn et al. (2008) demonstrated that quercetin suppressed adipogenesis and improved metabolic regulation in mature 3T3-L1 adipocytes through modulation of AMPK and MAPK signaling pathways. Arias et al. (2018) reported that quercetin metabolites restored GLUT4 expression, reduced lipid accumulation, and improved insulin signaling in insulin-resistant adipocyte models. In another study, Kobori et al. (2020) showed that quercetin glycosides enhanced GLUT4 translocation through activation of Ca2+/calmodulin-dependent protein kinase kinase-β (CaMKKβ)/AMPK signaling pathways, thereby improving hyperglycemia and insulin sensitivity in obese mice. Additionally, flavonoid compounds such as 7,8-dihydroxyflavone have been shown to improve insulin responsiveness in 3T3-L1 adipocyte coculture models by suppressing JNK and NF-κB-mediated inflammatory activation (Park et al., 2019). These findings collectively suggest that flavonoids can simultaneously modulate oxidative stress, inflammatory signaling, and insulin-sensitive metabolic pathways. Europetin-3-O-rhamnoside, a naturally occurring flavonoid glycoside structurally related to quercetin derivatives, has recently gained attention for its potential antioxidant and metabolic regulatory activities. However, its effects on adipocyte insulin resistance and insulin-sensitive signaling pathways remain largely unexplored. Considering the central role of oxidative stress and inflammatory signaling in adipocyte dysfunction, Europetin-3-O-rhamnoside may represent a promising therapeutic candidate capable of restoring insulin signaling and reducing oxidative injury under hyperglycemic conditions. Therefore, the present study aimed to investigate the antidiabetic potential of Europetin-3-O-rhamnoside in high glucose-induced insulin-resistant 3T3-L1 adipocytes, with particular emphasis on its effects on IR, IRS, PI3K, AKT, GLUT4, adiponectin, oxidative stress, and inflammatory signaling pathways. The findings of this study may provide mechanistic insight into the therapeutic potential of flavonoid-

based compounds 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.

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

Cell viability

The cell viability assay demonstrated that Europetin-3-O-Rhamnoside exerted a protective effect against high glucose (HG)-induced cytotoxicity in 3T3-L1 adipocytes. The control group maintained 100% cell viability, whereas HG exposure at 5 µg significantly reduced viability to nearly 70%, indicating glucose-induced cellular stress and metabolic impairment. Treatment with increasing concentrations of Europetin-3-O-Rhamnoside gradually restored cell viability, with HG+10 µg and HG+20 µg groups showing approximately 80% and 90% viability, respectively, suggesting a dose-dependent cytoprotective effect. The metformin-treated group exhibited the highest recovery, approaching normal control levels (~96%), confirming its established insulin-sensitizing activity. These findings indicate that Europetin-3-O-Rhamnoside improves adipocyte survival under hyperglycemic conditions and may enhance insulin sensitivity through modulation of GLUT-4 translocation and adiponectin expression.

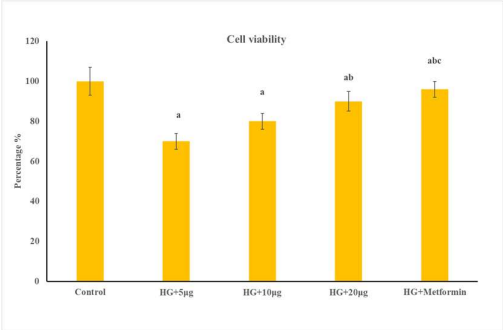


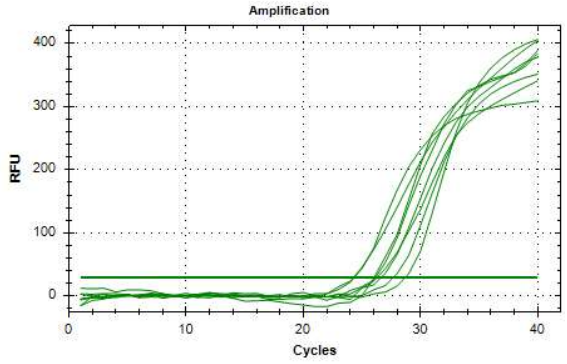
Figure 1. Effect of Europetin-3-O-Rhamnoside on cell viability in high glucose-induced 3T3-L1

adipocytes. High glucose exposure significantly reduced adipocyte viability, while treatment with Europetin-3-O-Rhamnoside restored viability in a dose-dependent manner. Metformin served as the positive control. Data are expressed as mean ± SD; different superscripts indicate statistically significant differences ($p < 0.05$).

Gene expression

IR mRNA Expression

The mRNA expression analysis of insulin receptor (IR) revealed a marked reduction in the HG+5 µg group compared to the control, indicating that high glucose conditions impaired insulin signaling in 3T3-L1 adipocytes. Treatment with Europetin-3-O-Rhamnoside at 10 µg moderately restored IR expression, while 20 µg treatment significantly enhanced IR mRNA levels close to the normal control and metformin-treated groups. The metformin group showed near-complete recovery of IR expression. These findings suggest that Europetin-3-O-Rhamnoside improves insulin sensitivity in a dose-dependent manner through restoration of insulin receptor expression.



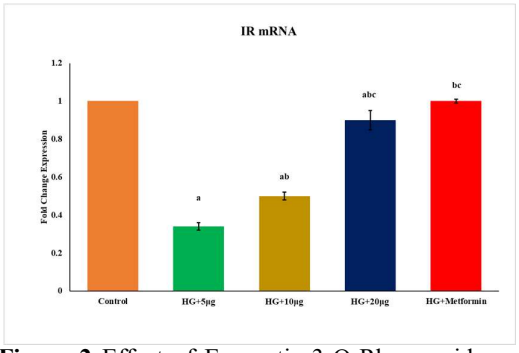
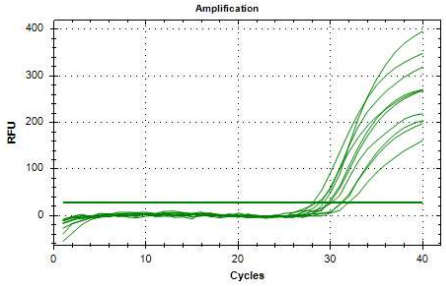
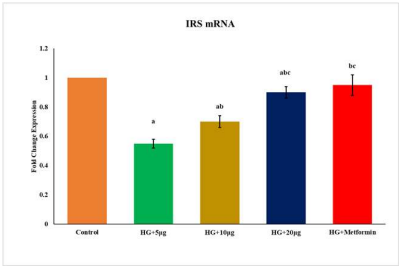


Figure 2 Effect of Europetin-3-O-Rhamnoside on IR mRNA expression in insulin-resistant 3T3-L1 adipocytes under high-glucose conditions. Data are expressed as mean ± SD, and different superscript letters indicate statistically significant differences between groups ($p < 0.05$).

IRS mRNA Expression

IRS mRNA expression was substantially decreased in the HG+5 µg group, confirming glucose-induced insulin resistance. Administration of Europetin-3-O-Rhamnoside progressively increased IRS expression with increasing concentrations. The HG+20 µg group exhibited expression levels comparable to the metformin-treated cells, indicating efficient recovery of insulin signaling pathways. The results demonstrate that Europetin-3-O-Rhamnoside positively regulates IRS transcription and may enhance downstream insulin-mediated glucose uptake.





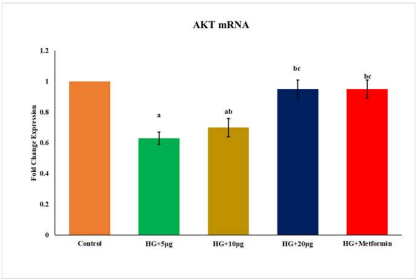


Figure 3 Effect of Europetin-3-O-Rhamnoside on IRS mRNA expression in high-glucose-induced insulin-resistant 3T3-L1 adipocytes. Values represent mean ± SD with statistical significance indicated by different superscripts ($p < 0.05$).

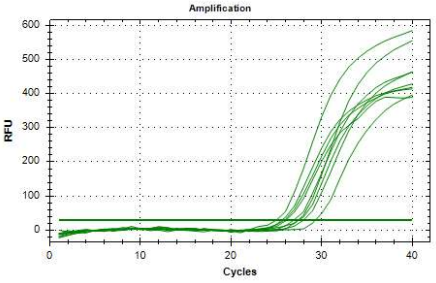
AKT mRNA Expression

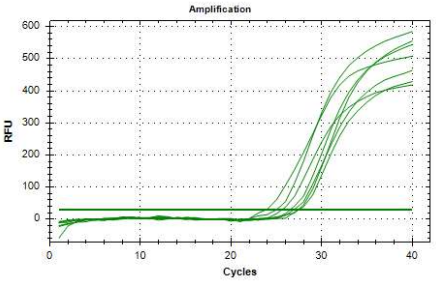
AKT mRNA levels were significantly suppressed under high-glucose conditions, suggesting impaired insulin signaling and glucose metabolism. Treatment with Europetin-3-O-Rhamnoside restored AKT expression in a concentration-dependent manner. The HG+20 µg and metformin-treated groups showed the highest AKT expression levels, approaching those of the control group. This indicates that Europetin-3-O-Rhamnoside activates the PI3K/AKT signaling pathway, thereby improving insulin responsiveness in insulin-resistant adipocytes.

Figure 4 Effect of Europetin-3-O-Rhamnoside on AKT mRNA expression in insulin-resistant adipocytes. Results are presented as mean ± SD, and groups with different superscript letters differ significantly at $p < 0.05$.

GLUT-4 mRNA Expression

High glucose (HG)-treated 3T3-L1 adipocytes exhibited a marked reduction in GLUT-4 mRNA expression compared to the control group, indicating impaired insulin-mediated glucose transport under hyperglycemic conditions. Treatment with Europetin-3-O-Rhamnoside significantly restored GLUT-4 expression in a dose-dependent manner. The 5µg treatment showed only a slight improvement, whereas 10 µg notably increased GLUT-4 levels, suggesting partial recovery of insulin sensitivity. The 20µg treatment further enhanced GLUT-4 expression close to normal control levels and showed efficacy comparable to metformin. Since GLUT-4 is a key glucose transporter responsible for cellular glucose uptake, these findings suggest that Europetin-3-O-Rhamnoside improves glucose utilization and insulin responsiveness in adipocytes.





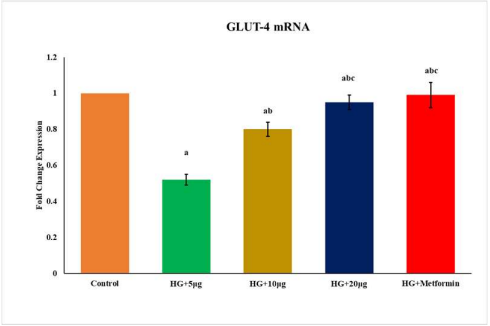
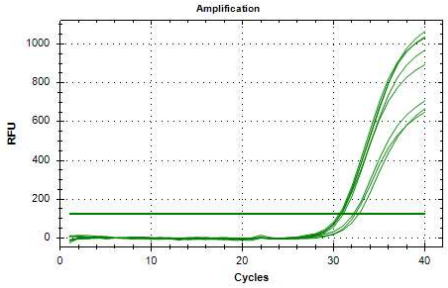


Figure 5 Effect of Europetin-3-O-Rhamnoside on GLUT-4 mRNA expression in HG-induced 3T3-L1 adipocytes. HG significantly reduced GLUT-4 expression, while treatment with Europetin-3-O-Rhamnoside dose-dependently restored its expression, comparable to metformin at 20 µg. Data are expressed as mean ± SD; a, b, and c indicate statistical significance at $p < 0.05$.

PI3K mRNA Expression

PI3K mRNA expression was significantly downregulated in HG-induced insulin-resistant adipocytes compared to the control group, reflecting disruption of the insulin signaling pathway. Treatment with Europetin-3-O-Rhamnoside significantly increased PI3K expression in a concentration-dependent manner. The 5µg treatment produced minimal recovery, while 10 µg and 20 µg treatments markedly elevated PI3K expression toward normal levels, with the highest dose showing effects comparable to metformin. As PI3K is a crucial mediator of insulin signal transduction and GLUT-4 activation, the observed upregulation indicates that Europetin-3-O-Rhamnoside enhances insulin signaling and may alleviate insulin resistance in 3T3-L1 adipocytes under hyperglycemic stress.



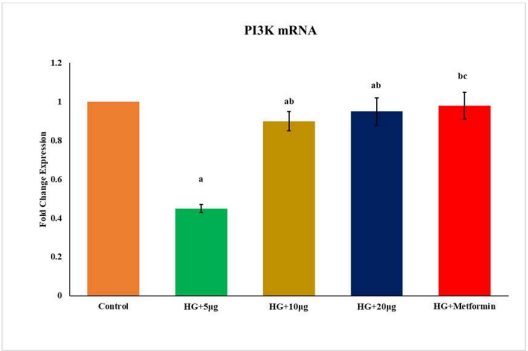


Figure 6 Effect of Europetin-3-O-Rhamnoside on PI3K mRNA expression in HG-induced 3T3-L1 adipocytes. PI3K expression was significantly suppressed under HG conditions and was restored following Europetin-3-O-Rhamnoside treatment in a dose-dependent manner. Data are presented as mean ± SD; a, b, and c indicate statistical significance at $p < 0.05$.

Effect of Europetin-3-O-Rhamnoside on oxidative stress and antioxidant biomarkers in

high-glucose-induced insulin-resistant 3T3-L1 adipocytes

The antioxidant biomarker analysis demonstrated that high-glucose exposure induced severe oxidative stress in 3T3-L1 adipocytes, as evidenced by decreased SOD, CAT, and GSH levels along with elevated H_2O_2 concentration in the HG+5 µg group compared to the control. Treatment with Europetin-3-O-Rhamnoside significantly improved antioxidant defense markers in a dose-dependent manner. The HG+20 µg group showed substantial restoration of SOD, CAT, and GSH levels while reducing H_2O_2 accumulation close to normal control values. Metformin treatment produced comparable protective effects. These findings indicate that Europetin-3-O-Rhamnoside exerts potent antioxidative activity and protects adipocytes against high-glucose-induced oxidative damage.

Biomarkers	Control	HG +5µg	HG+ 10µg	HG+ 20µg	HG+ Metformin
SOD (ng/mL)	25± 1.7	6±0.3	16±0.9	19±0.8	22±1.1
CAT (Pg/mL)	40± 2.5	19±1.1	29±1.2	30±1.9	39±1.9
H2O2 (µMol/L)	59± 3.2	125± 1.5	80±3.2	65±2.7	59±3.2
GSH (Pg/mL)	29± 0.2	12±0.8	19±0.8	22±1.7	25±1.7

Table 1 Effect of Europetin-3-O-Rhamnoside on oxidative stress biomarkers in high-glucose-induced insulin-resistant 3T3-L1 adipocytes. Values are expressed as mean ± SD. SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione; H_2O_2: Hydrogen peroxide.

Discussion

The present study demonstrates that Europetin-3-O-rhamnoside effectively attenuates high glucose-induced insulin resistance in 3T3-L1 adipocytes through modulation of insulin-sensitive signaling pathways and restoration of cellular redox balance. High glucose exposure significantly suppressed the expression of IR, IRS, PI3K, AKT, and GLUT4, while simultaneously reducing antioxidant defenses including SOD, CAT, and GSH and elevating H2O2 levels. Treatment with Europetin-3-O-rhamnoside markedly reversed these alterations in a dose-dependent manner, indicating recovery of insulin signaling, improvement of glucose transport mechanisms, and attenuation of oxidative stress-mediated cellular dysfunction. These findings suggest that Europetin-3-O-rhamnoside may improve adipocyte insulin responsiveness through coordinated regulation of metabolic and antioxidant pathways. Insulin resistance in adipocytes is now understood as a complex metabolic disorder involving disruption of multiple interconnected signaling pathways rather than a simple impairment in glucose transport. Under normal physiological conditions, insulin binding to IR activates IRS proteins and downstream PI3K/AKT signaling, which promotes GLUT4 translocation to the plasma membrane and facilitates glucose uptake. Defects in this pathway contribute significantly to impaired glucose utilization and the development of type 2 diabetes mellitus (Klip et al., 2019). Czech (2017) further described insulin resistance as a consequence of altered insulin receptor signaling, lipid overload, mitochondrial dysfunction, and inflammatory stress. In agreement with these concepts, the present study demonstrated that high glucose markedly downregulated IR, IRS, PI3K, AKT, and GLUT4 expression, confirming severe impairment of canonical insulin-signaling pathways in insulin-resistant adipocytes.

Among these biomarkers, GLUT4 restoration is particularly important because GLUT4 is the major insulin-responsive glucose transporter in adipose and skeletal muscle tissues. Impaired GLUT4 expression and defective translocation are considered key molecular events contributing to hyperglycemia and metabolic dysfunction in insulin-resistant states (Petersen & Shulman, 2018). The observed dose-dependent increase in GLUT4 expression following Europetin-3-O-rhamnoside treatment therefore strongly indicates enhanced insulin responsiveness and improved glucose transport capacity. Similar findings have been reported for quercetin-derived flavonoids, which restored GLUT4 and AKT signaling in insulin-resistant adipocyte models (Arias et al., 2018). Kobori et al. (2020) also demonstrated that quercetin glycosides enhanced GLUT4 translocation and improved hyperglycemia through activation of AMPK-associated pathways in obese diabetic mice. These findings collectively support the hypothesis that Europetin-3-O-rhamnoside exerts insulin-sensitizing effects through restoration of the IR/IRS/PI3K/AKT/GLUT4 signaling axis. Oxidative stress is recognized as one of the major pathogenic factors contributing to insulin resistance and adipocyte dysfunction. Chronic hyperglycemia promotes excessive generation of reactive oxygen species (ROS), which impair mitochondrial activity, activate inflammatory signaling pathways, and interfere with insulin receptor-mediated glucose uptake (Evans et al., 2002). Furukawa et al. (2004) reported that oxidative stress in adipose tissue plays a central role in the progression of metabolic syndrome and insulin resistance through activation

of stress-sensitive kinases and inflammatory mediators. Similarly, Houstis et al. (2006) demonstrated that ROS directly induce insulin resistance by suppressing insulin-sensitive signaling pathways. In the present study, high glucose exposure markedly reduced SOD, CAT, and GSH levels while increasing H2O2 concentration, indicating severe oxidative stress-mediated cellular injury. However, treatment with Europetin-3-O-rhamnoside significantly restored antioxidant enzyme levels and reduced oxidative burden, demonstrating potent antioxidant activity.

The improvement in antioxidant biomarkers observed in this study is mechanistically important because oxidative stress and insulin signaling are closely interconnected processes. A redox-impaired adipocyte is more susceptible to inflammatory kinase activation, mitochondrial dysfunction, and suppression of insulin-sensitive signaling pathways. Restoration of antioxidant defenses may therefore relieve oxidative inhibition of insulin signaling and improve metabolic homeostasis. The increase in SOD, CAT, and GSH levels together with reduced H2O2 suggests that Europetin-3-O-rhamnoside may act upstream of, or parallel with, insulin-signaling restoration by attenuating oxidative stress. Similar effects have been reported for quercetin and related flavonoids, which improve insulin resistance while simultaneously reducing oxidative stress and inflammation (Li et al., 2016). Rivera et al. (2024) also concluded that quercetin-derived flavonoids exert insulin-sensitizing effects through antioxidant and anti-inflammatory mechanisms. Fernández-Sánchez et al. (2011) further emphasized that oxidative stress and chronic inflammation are closely linked in obesity-associated metabolic disorders, thereby supporting the therapeutic relevance of antioxidant compounds in insulin resistance. The PI3K/AKT pathway plays a critical role in insulin-mediated glucose uptake, glycogen synthesis, and cellular metabolism. Activation of PI3K stimulates AKT phosphorylation, which subsequently promotes GLUT4 translocation and enhances glucose utilization. Suppression of PI3K and AKT under diabetic conditions contributes significantly to impaired insulin responsiveness (Samuel & Shulman, 2016). In the present study, Europetin-3-O-rhamnoside restored PI3K and AKT expression, indicating recovery of insulin-sensitive signaling pathways. Similar findings have been reported for flavonoid-treated diabetic models, where restoration of PI3K/AKT signaling improved glucose uptake and metabolic regulation (Hanhineva et al., 2010). Lee et al. (2019) additionally demonstrated that quercetin glycosides improved glucose metabolism through activation of AMPK and insulin-sensitive signaling pathways, producing effects comparable to metformin.

AMPK-associated signaling pathways may also contribute to the observed metabolic improvements. AMPK functions as a central cellular energy sensor regulating glucose uptake, fatty acid oxidation, mitochondrial metabolism, and insulin sensitivity (Hardie et al., 2012). Activation of AMPK promotes GLUT4 translocation and improves insulin responsiveness under hyperglycemic conditions. Kahn et al. (2005) described AMPK as a major therapeutic target for metabolic disorders because of its ability to regulate multiple pathways involved in energy homeostasis. Therefore, the restoration of insulin-sensitive signaling observed in the present study may partly involve modulation of AMPK-associated metabolic pathways by Europetin-3-O-rhamnoside. Inflammation is another major contributor to insulin resistance and adipocyte dysfunction. Hyperglycemia-induced ROS production activates inflammatory mediators such as NF-κB and JNK, which impair insulin receptor signaling and worsen metabolic dysfunction (Hotamisligil, 2006). Several flavonoids have been shown to suppress inflammatory signaling and restore insulin sensitivity. Park et al. (2019) demonstrated that 7,8-dihydroxyflavone improved insulin responsiveness in adipocytes by inhibiting JNK and NF-κB activation. Therefore, the antioxidant effects of Europetin-3-O-rhamnoside observed in the present study may additionally contribute to attenuation of inflammation-associated insulin resistance. Despite these promising findings, several limitations should be acknowledged. The present investigation primarily evaluated transcript-level changes in insulin-signaling molecules. Although restoration of IR, IRS, PI3K, AKT, and GLUT4 expression strongly suggests improved insulin sensitivity, mRNA expression alone does not confirm protein abundance, phosphorylation status, or functional pathway activation. Additional analyses involving Western blotting for phosphorylated AKT (p-AKT), PI3K, and GLUT4 proteins, GLUT4 membrane translocation assays, and glucose uptake measurements would substantially strengthen the mechanistic interpretation. Furthermore, pathway inhibition studies using PI3K or AKT inhibitors are necessary to determine whether Europetin-3-O-rhamnoside directly acts through canonical insulin-signaling pathways or indirectly improves signaling through antioxidant-mediated mechanisms. Finally, the current findings are limited to an in vitro adipocyte model, and further in vivo studies are required to evaluate the bioavailability, pharmacokinetics, and therapeutic efficacy of the compound under physiological conditions. Overall, the present study demonstrates that Europetin-3-O-rhamnoside ameliorates high glucose-induced insulin resistance in 3T3-L1 adipocytes through restoration of IR/IRS/PI3K/AKT/GLUT4-associated signaling

pathways and enhancement of antioxidant defense systems. The compound effectively reduced oxidative stress, improved insulin-sensitive signaling, and restored adipocyte metabolic responsiveness under hyperglycemic conditions. These findings suggest that Europetin-3-O-rhamnoside may serve as a promising natural flavonoid candidate for the development of novel therapeutic strategies targeting insulin resistance and type 2 diabetes mellitus.

Conclusion

The present study demonstrates that Europetin-3-O-rhamnoside possesses significant antidiabetic potential against high glucose-induced insulin resistance in 3T3-L1 adipocytes through modulation of insulin-sensitive signaling pathways and oxidative stress-associated mechanisms. Treatment with Europetin-3-O-rhamnoside effectively restored the expression of IR, IRS, PI3K, AKT, and GLUT4, indicating improvement of insulin signaling and glucose transport capacity. In addition, the compound enhanced antioxidant defense systems by increasing SOD, CAT, and GSH levels while reducing H2O2 accumulation, thereby alleviating oxidative stress-mediated cellular dysfunction. These findings suggest that the protective effects of Europetin-3-O-rhamnoside may involve restoration of the PI3K/AKT/GLUT4 signaling axis together with attenuation of oxidative stress in insulin-resistant adipocytes. However, the present study is limited to transcript-level observations in an in vitro model, and further investigations involving protein expression, GLUT4 translocation assays, glucose uptake studies, pathway inhibition experiments, and in vivo diabetic models are necessary to validate the precise molecular mechanisms and therapeutic efficacy of the compound. Overall, Europetin-3-O-rhamnoside represents a promising natural flavonoid candidate for the development of novel therapeutic strategies targeting insulin resistance and type 2 diabetes mellitus.

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