



Comparative Effects of *Nigella sativa* and Thymoquinone on Glucose Metabolism in a High Glucose-induced Renal Cell Line

A. Usta¹, S. Çetin², V. Yüksek³, S. Dede⁴

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ABSTRACT

Background: A significant side effect of type 2 diabetes mellitus is diabetic nephropathy (DN), which is typified by poor glucose homeostasis and insulin resistance in renal tissues. Thymoquinone (TQ), the active ingredient in *Nigella sativa* (NS), has been shown to have antioxidant and antidiabetic properties. However, nothing is known about their relative effects on renal glucose metabolism in hyperglycemic situations. The objective of this work was to examine and contrast the regulatory effects of NS and TQ on insulin signaling pathways and glucose transporter expression in NRK-52E renal epithelial cells grown in high-glucose environments.

Methods: TQ (10 µM) or NS extract (0.5 µg/ml) were applied to NRK-52E cells after they were subjected to high glucose (246 mM). RT-qPCR was used to measure the mRNA expression levels of insulin signaling components (Ir, Irs1, Pi3k, Ampk, Gsk3β) and glucose transporters (Glut1, Glut2, Glut3, Glut5, SglT2). Western blot was used to measure the protein levels of Glut2, Glut3, SglT2 and Irs1. The Duncan test ($p < 0.05$) and one-way ANOVA were used to determine statistical significance.

Result: Ir, Irs1, Pi3k and Ampk levels were downregulated and Glut1, Glut2, Glut3 and SglT2 expression was markedly elevated by high glucose exposure. These changes were reversed by TQ and NS therapy, with TQ having a stronger effect. TQ increased the levels of Irs1 and Pi3k while more successfully suppressing the expression of SglT2 and Glut2. The transcriptional alterations were confirmed by Western blot results. TQ and NS improved insulin signaling pathways and modulated glucose transporter expression to protect renal cells. These results confirm that TQ and NS are safe to use as possible therapeutic agents to treat diabetic nephropathy.

Key words: Diabetic nephropathy, Glucose metabolism, Glut, Insulin signaling, *Nigella sativa*, NRK-52E, SGLT2, Thymoquinone.

INTRODUCTION

Diabetic nephropathy is an important side effect of diabetes that can lead to chronic kidney failure and significantly reduce a patient's quality of life. Vascular injury in intraglomerular arterioles is the main way that persistent hyperglycemia causes renal failure. It is thought to affect about 15% of patients with type 2 diabetes and 35% of those with type 1 diabetes (Eraslan Farisoğlu *et al.*, 2026; Özcan and Kaya, 2025; Raj and Rani, 2024).

Diabetes raises the risk of organ failure and persistent tissue damage due to impaired glucose regulation (Keembiyehetty *et al.*, 2006). Cellular function depends on the movement of glucose across cell membranes, which is accomplished by either active or assisted diffusion. Tissue-specific expression of sodium-glucose cotransporters (SglTs) and glucose transporters (Gluts) occurs in the kidney. SglT2, which is found on the apical membrane of proximal tubular cells, is principally in charge of sodium-dependent glucose reabsorption, which uses basolateral Glut transporters to move glucose into the bloodstream (Norton *et al.*, 2017; Bailey *et al.*, 2010).

Gluts comprise 14 isoforms that are categorized into three classes and are widely expressed in mammalian cells (Augustin, 2010; Mueckler *et al.*, 2013). Hormonal, metabolic and oxidative stress-related factors impact their

¹Van Yuzuncu Yil University, Faculty of Science, Department of Chemistry, Van, Türkiye.

²Ankara Yıldırım Beyazıt University, Vocational School of Health Services, Department of Veterinary Medicine, Ankara, Türkiye.

³Van Yuzuncu Yil University, Ozalp Regional High School, Van, Türkiye.

⁴Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Biochemistry Department, Van, Türkiye.

Corresponding Author: A. Usta, Van Yuzuncu Yil University, Faculty of Science, Department of Chemistry, Van, Türkiye. Email: ayseusta@yyu.edu.tr

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expression, which varies according to tissue type and pathological situations such diabetes, ischemia and cancer (Rizzo *et al.*, 2013; Medina and Owen, 2002).

Insulin primarily uses the Pi3k/Akt and MAPK signaling pathways to control glucose homeostasis by activating the insulin receptor (Ir) and insulin receptor substrate (Irs) (Cardoso, 2009). Irs1 phosphorylation is essential for Pi3k activation, which increases glucose absorption through

Glut translocation, especially Glut4 (Lee *et al.*, 2012). Additionally, Ampk is a key target for treatment in type 2 diabetes and plays a crucial role in glucose metabolism (Bijland *et al.*, 2013; Manna *et al.*, 2017; Park *et al.*, 2018).

Glut2-mediated glucose absorption in pancreatic β -cells raises intracellular ATP levels, which in turn cause membrane depolarization and calcium influx, which in turn stimulate insulin production (Champe and Harvey 2007). Furthermore, a crucial regulator of glycogen metabolism, glycogen synthase kinase 3 beta (Gsk3 β), is still active in diabetes, which inhibits glycogen synthesis and lowers cellular glucose absorption.

In diabetic patients, *Nigella sativa* (NS) has been shown to enhance inflammatory indicators, oxidative stress, insulin resistance, lipid profiles and glycemic management. Additionally, it improves antioxidant capacity and β -cell activity (Shan *et al.*, 2011; O'Neill, 2013; Hamdan *et al.*, 2019; Kaatabi *et al.*, 2015; Chatterjee *et al.*, 2025; Heshmati *et al.*, 2015). The primary bioactive component of NS, thymoquinone (TQ), has shown protective properties in diabetic mice by lowering inflammation, apoptosis and oxidative stress, in part by modifying the Pi3k/Akt pathway (Liu *et al.*, 2016; He *et al.*, 2023; Usta and Dede, 2017).

Despite these results, the effects of NS and TQ on renal glucose metabolism are still unknown and changes in glucose transporters under metabolic diseases are not completely understood. Therefore, by examining the mRNA and protein expression levels of important genes involved in glucose metabolism, such as Glut1, Glut2, Glut3, Glut5, SglT2, Ir, Irs1, Pi3k, Ampk and Gsk3 β , this study sought to determine how NS and TQ affected renal proximal tubular cells (NRK-52E) cultured under high-glucose conditions.

MATERIALS AND METHODS

Reagents and chemicals

Thymoquinone (TQ) and D-glucose were acquired from Sigma-Aldrich (USA). Western blot kits and antibodies were purchased from Affinity Biosciences (USA). The ABT 2x SYBR Green Master Mix (Türkiye) was used for RT-qPCR studies.

Cell culture

NRK-52E (ATCC® CRL-1571TM), a rat kidney epithelial cell line, was grown in RPMI-1640 media supplemented with 1% L-glutamine, 1% penicillin/streptomycin and 10% fetal bovine serum (Capricorn, Germany). The cells were kept in a humidified environment with 5% CO₂ at 37°C (Esco CelCulture CO₂, Singapore). The Van Yuzuncu Yil University Scientific Research Projects Coordination Unit project TYD-2020-8582 provided funding for all of the experiments, which were carried out in 2022 at the Cell Culture and Molecular Biology Laboratory in the Biochemistry Department of the Faculty of Veterinary Medicine at Van Yuzuncu Yil University.

Preparation of solutions

In order to make *Nigella sativa* (NS) extract, 70% of the powdered seeds were extracted using methanol, filtered, the solvent was evaporated, the mixture was lyophilized and it was then stored at -20°C (Meziti *et al.*, 2012). TQ and NS stock solutions were made in DMSO (Panreac Applichem, Spain) (<0.05%) and then diluted in culture medium. As previously stated, amounts of glucose (0-400 mM), NS (0-200 μ g/ml) and TQ (0-100 μ M) were prepared (Gümüş *et al.*, 2018; Çelik, 2013; Usta *et al.*, 2024a).

Table 1: Study groups based on the administration of glucose, TQ and NS to NRK-52E cells.

Groups	Application
Control	C-Cell medium
Thymoquinone	TQ-Thymoquinone (proliferative concentration -10 μ M)
<i>Nigella sativa</i>	NS- <i>Nigella sativa</i> (proliferative concentration -0,5 μ g/ml)
High glucose	HG-Glucose (IC ₅₀ -246 mM)
High glucose +TQ	GT-Glucose (IC ₅₀ -246 mM) + Thymoquinone (prolif. conc.-10 μ M)
High glucose +NS	GN-Glucose (IC ₅₀ -246 mM) + <i>Nigella sativa</i> (prolif. conc.-0,5 μ g/ml)

Table 2: Target gene primers' base sequence.

	F: 5'-3'	R: 5'-3'
Glut1	ATAGGGGTCCAGGCTCCATT	TCCGAGGTCCTTCTCATGGT
Glut2	CTTCCAGTACATTGCGGACTT	GAATTCCATTTGTACAGCGGC
Glut3	CCACGCTCTGGTCGTTATGT	ATGGAGTTGCGTCTGCCAA
Glut5	CTACCCTTGACAGCAACGA	GAGCCAAAGGCAGCTAGGAA
SglT2	ATATCTACACACGCCTGCGG	AAAGACAGCGGACACTGGAG
Irs1	GTTGAGAGCGGTGGTGTA	GCTTGTGCTGGGGATCTTCT
Ampk	CATGGCTGAGAAGCAGAAGC	CTGCCACTTTATGGCCTGTC
Ir	CAGAAGAGAGGTGCAGCGTG	TCCTTTGGCTCTTGCCACAT
Gsk3 β	ACCACAGAACCTCTTGCTGG	CTAGACGTGTAATCGGTGGCT
Pi3k	GGAGAACTATGAACAACCTGTG	CATCTTCCAGTAACGTAGGCAG
Actb	CTCCTCAAGGATGGCACC	GCTCATTGTAGAAAGTGTGGT

MTT assay

Following the seeding of 1×10^4 cells per well in 96-well plates, the cells were incubated overnight. The MTT assay was used to evaluate cell viability following treatment. The formazan crystals made by metabolically active cells were dissolved and the absorbance at 570 nm was determined (Biochrom, Anthos Zenyth 200). The viable cell percentage was contrasted with the control (Usta *et al.*, 2024b; Meerloo *et al.*, 2011).

Experimental design

Treatment groups were established based on different concentrations of glucose, TQ and NS, as indicated in Table 1.

RNA isolation and cDNA synthesis

Each group received a 24-hour treatment after being planted with about 8×10^5 cells. Total RNA was isolated using Trizol

reagent (GeneAll, Korea) and its purity was assessed by spectrophotometric analysis (Chomczynski and Mackey, 1995). The manufacturer's instructions were followed for cDNA synthesis using a commercial kit (ABT High Capacity, Türkiye).

RT-qPCR analysis

The findings of RT-qPCR investigation of Sglt2, Glut1, Glut2, Glut3, Glut5, Ir, Irs1, Gsk3 β , Pi3k and Ampk gene expression levels are displayed in Table 2 (Qiagen, Germany).

Using β -actin as the internal reference, relative expression levels were determined using the $2^{-\Delta\Delta Ct}$ approach (Livak and Schmittgen, 2001) (Table 3).

Western blot analysis

After the proteins were extracted (GeneAll, Korea) and quantified using the Bradford procedure (ABT Biosciences,

Table 3: RT-qPCR reaction conditions.

Reaction content	For an example	Reaction cycle
Mastermix (2X)	10 μ	1-95°C 5' denaturation
Primer forward	0.5 μ l	2-95°C 20"
Reverse	0.5 μ l	3-50-60°C 60"
dH ₂ O	8 μ l	(varies by gene)
cDNA	1 μ l	Melting Curve
Total	20 μ l	Ramp: 50-99 (1 degree increase) 90°C 5"

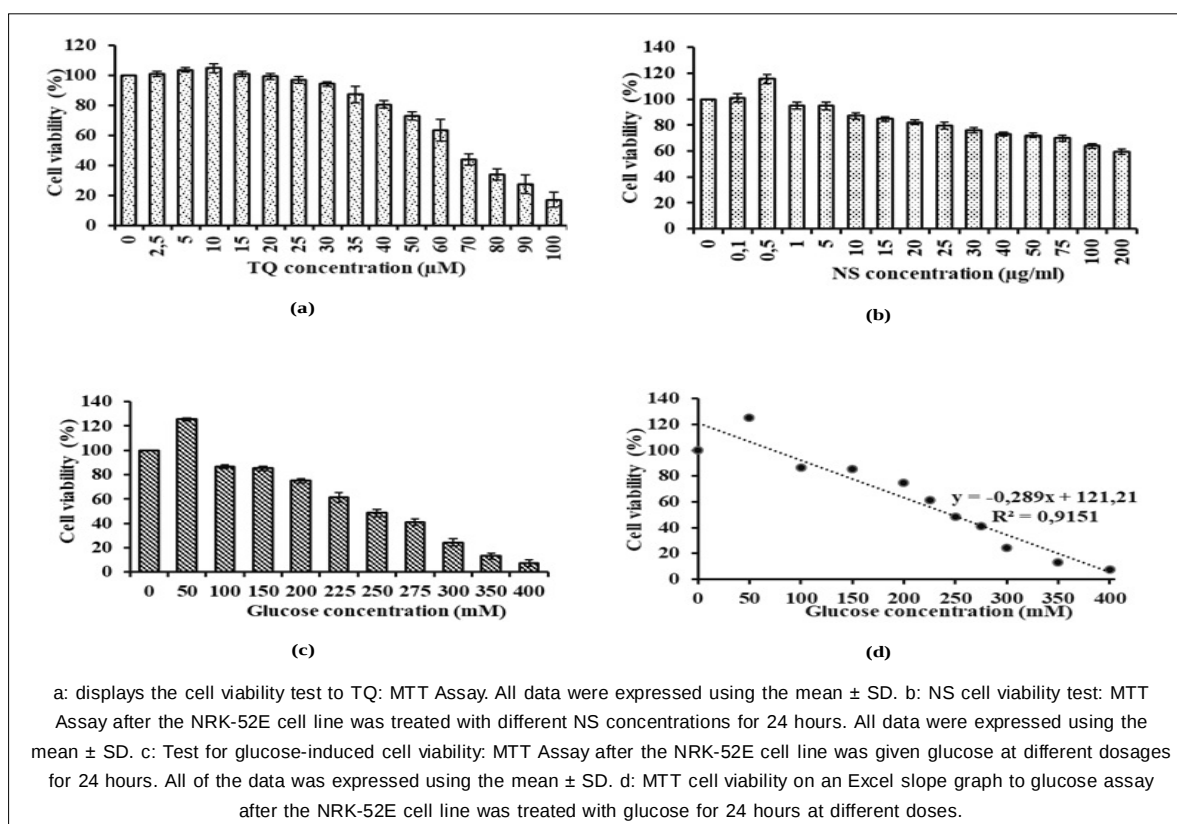


Fig 1: After administering different TQ, NS and glucose concentrations to the NRK-52E cell line for 24 hours.

Türkiye), they were separated using SDS-PAGE. After being moved to nitrocellulose membranes, samples were incubated with primary and secondary antibodies. Protein bands were seen using a chemiluminescent substrate and analysis was done using ImageJ software.

Statistical analysis

SPSS 22.0 was used for statistical analysis (IBM, USA). The Duncan test was used after one-way ANOVA and Kruskal-Wallis to assess group differences. Mean $\pm$ standard deviation was used to express the data and $p < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Effects of TQ, NS and glucose on cell viability

TQ, NS and glucose concentrations that were cytotoxic and proliferative in NRK-52E cells were measured using the MTT test (Fig 1). The best proliferative doses were determined to be TQ (10 μ M) and NS (0.5 μ g/ml), which both markedly improved cell viability ($p < 0.05$). In the

experiments that followed, the cytotoxic concentration of glucose was established to be 246 mM based on its IC_{50} value.

Effects on glucose transporter gene expression

High glucose considerably increased the expression of Glut1, Glut2, Glut3 and SglT2 in comparison to the control, according to RT-qPCR study (Fig 2). These increases were reversed by TQ and NS treatment, bringing expression levels back to nearly control levels. Nonetheless, the treated groups' Glut2 expression was still lower than the control. All experimental groups had lower levels of Glut5 expression than the control.

Effects on Ir and Irs1 expression

Ir and Irs1 were downregulated in comparison to the control in high glucose circumstances (Fig 3). Both genes' expression was elevated by TQ and NS treatments in comparison to the glucose group. Irs1 levels were brought back to almost control levels in treatment groups, but Ir expression remained below control.

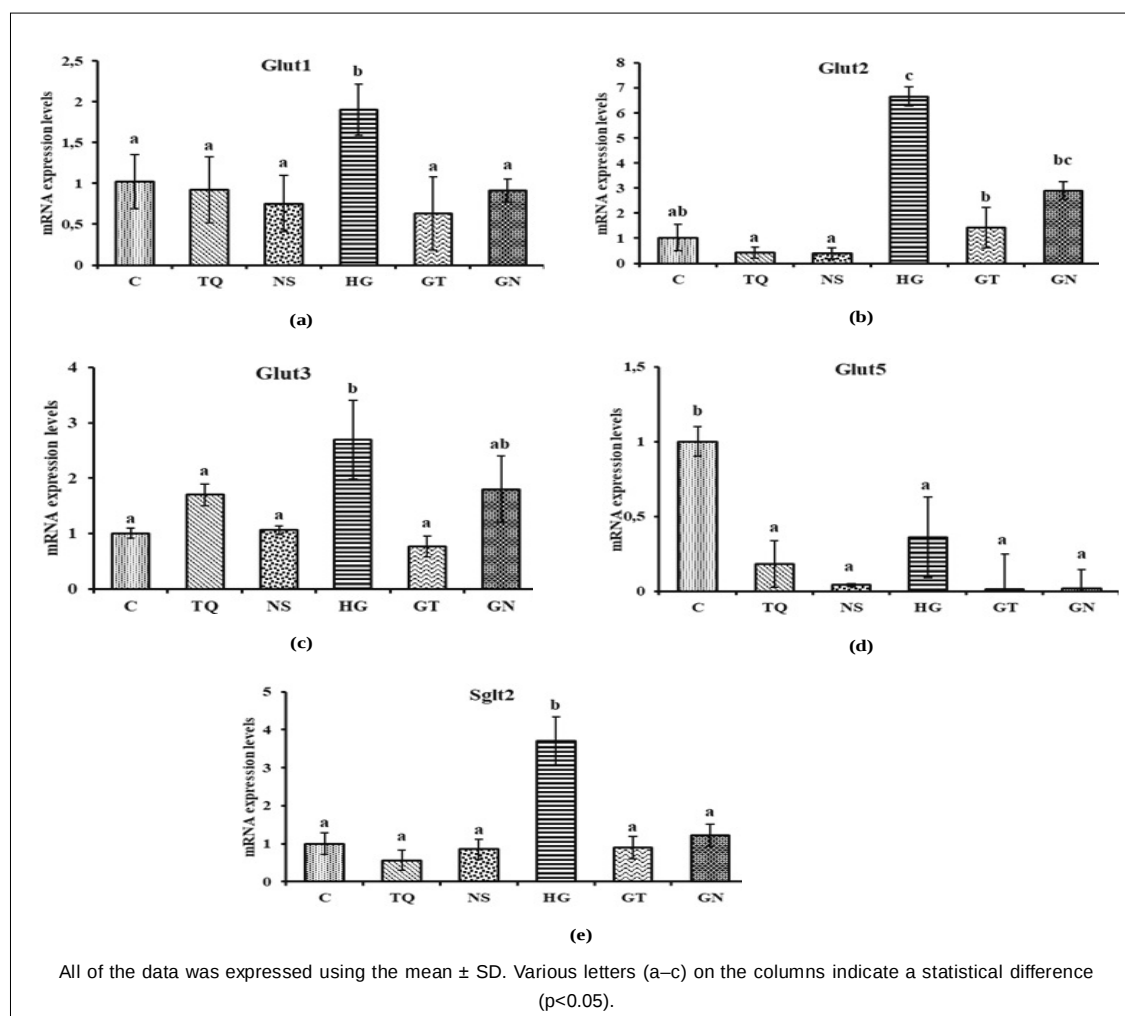


Fig 2: (a-e) Glut1, Glut2, Glut3, Glut5 and SglT2 mRNA transcription levels in NRK-52E cells after 24 hours.

Effects on Pi3k, Gsk3 β and Ampk expression

Under high glucose circumstances, Pi3k and Ampk expression levels decreased; however, after TQ and NS treatment, they either increased or were recovered (Fig 4). While there was no discernible difference between the treatment groups and the control, Gsk3 β expression was increased in the glucose group. On the other hand, Gsk3 β expression was decreased by TQ and NS alone.

Protein expression analysis

The glucose-treated group had higher amounts of Glut2 protein than the control group, according to Western blot analysis and these levels dropped after TQ and NS treatment (Fig 5). In general, all groups had lower levels of other target proteins than the control. These results most

likely represent early transcriptional responses at 24 hours, since longer exposure times may be necessary for protein-level alterations (48-72 h).

In this investigation, NRK-52E cells subjected to rising glucose concentrations for a full day demonstrated a dose-dependent decline in viability, with an IC_{50} of 246 mM, simulating advanced hyperglycemic stress in line with other findings. (Liu *et al.*, 2016; Gholamnezhad *et al.*, 2016). This method allowed for the assessment of renal tubular cells' impaired insulin signaling and glucose-induced cytotoxicity.

Thymoquinone (TQ) is one of the main bioactives found in *Nigella sativa* (NS) extract. Both have been shown to have anti-inflammatory, anti-apoptotic, antioxidant and antidiabetic properties (Sangi *et al.*, 2015; Ali and Blunden, 2003).

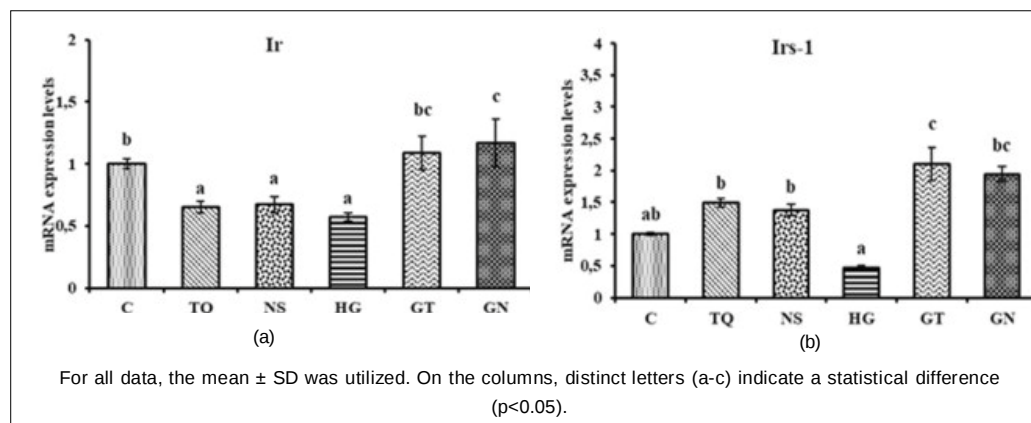


Fig 3: (a-b) Irs1 and Ir mRNA transcription levels in NRK-52E cells after 24 hours.

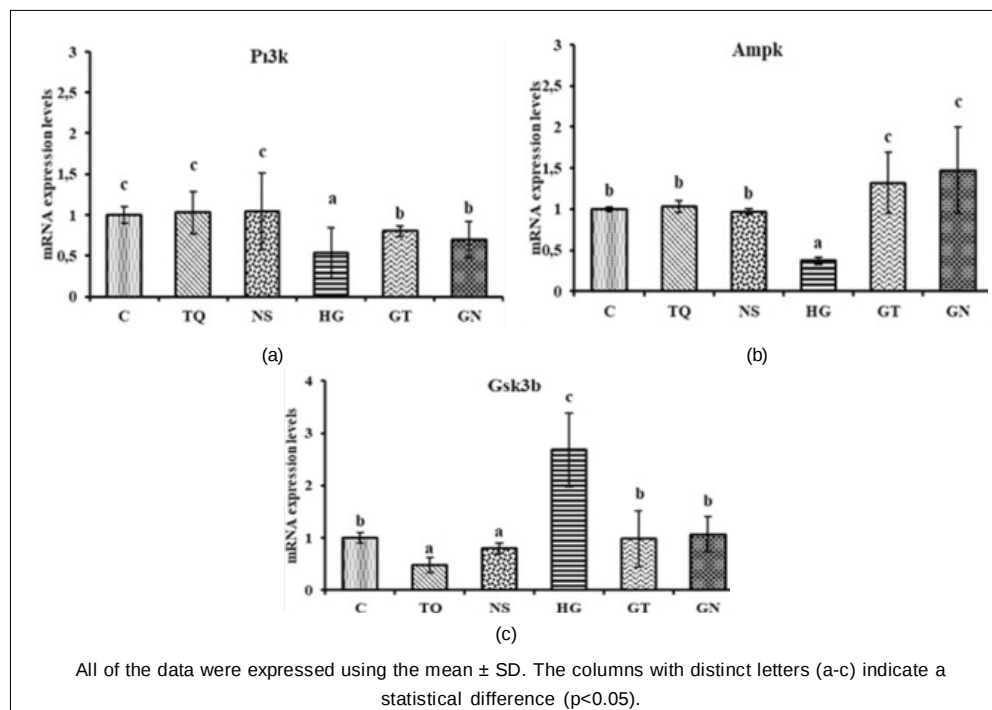


Fig 4: (a-c) Pi3k, Gsk3 β and Ampk mRNA transcription levels at 24 hours in NRK-52E cells.

Sgt2 reabsorbs approximately 90% of filtered glucose in proximal tubules, while Glut1/Glut2 facilitates translocation to the circulation. SglT and Glut transporters control glucose uptake (Bell *et al.*, 1990; Iancu *et al.*, 2022; Watson and Pessin, 2001; Umino *et al.*, 2018; Girard, 2017; Marks *et al.*, 2003; Mather and Pollock, 2011). Hyperglycemia enhances transporter expression and renal glucose reabsorption, which exacerbates glucose toxicity (Abdul-Ghani *et al.*, 2011; Holman, 2020; Vestri *et al.*, 2001; Rahmoune *et al.*, 2005; DeFronzo, 2009; Liu *et al.*, 2012). High glucose increased Glut1, Glut2, Glut3 and SglT2 in our investigation, which is consistent with the literature. However, TQ and NS therapy brought these levels down to almost control levels. All groups showed low levels of Glut5, which is consistent with its restricted role in the kidneys (Adeshara *et al.*, 2017; Gnudi *et al.*, 2007; Brosius and Heilig, 2005; Vallon *et al.*, 2011; Sugawara-Yokoo *et al.*, 1999; Rand *et al.*, 1993; Inukai *et al.*, 1993).

Insulin signaling via the downstream Pi3k/Akt pathway and Ir/Irs1 is essential for maintaining glucose homeostasis. Elevated glucose inhibited the production of Ir and Irs1, which hindered insulin signaling and decreased the absorption of glucose. The expression of Pi3k/Akt and Irs1 was restored by TQ and NS, indicating improved glycogen production and insulin signaling (Tiwari *et al.*, 2013; Peng and He, 2018; Mokashi *et al.*, 2017; Linnemann *et al.*, 2014; Gatica *et al.*, 2013; Lay *et al.*, 2024; Mima *et al.*, 2011; Mima *et al.*, 2023; Lay and Coward, 2018; Chen *et al.*, 2022).

Under high glucose, AMPK, a crucial regulator of energy balance, was inhibited, which increased oxidative stress and insulin resistance (Coughlan *et al.*, 2014; Soetikno *et al.*, 2013; Han *et al.*, 2021; Welsh *et al.*, 2010). TQ and NS improved glucose utilization and reversed insulin resistance via restoring AMPK expression. In a similar vein, Gsk3 β , which rises in diabetes and prevents the synthesis of glycogen, was increased in high glucose but repressed

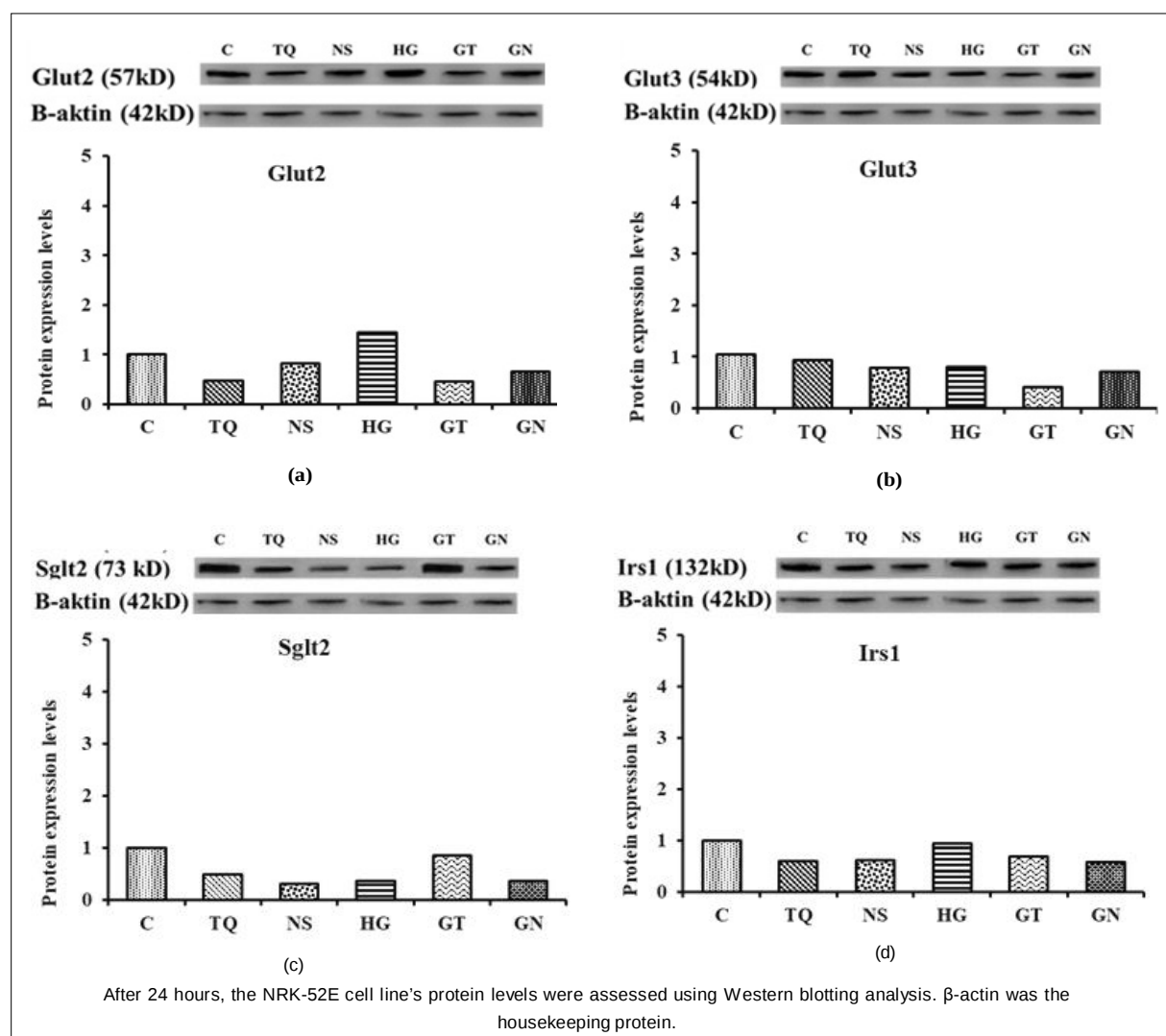


Fig 5: (a-d) Glut2, Glut3, SglT2 and Irs1 protein translation levels in the NRK-52E cell line after 24 hours.

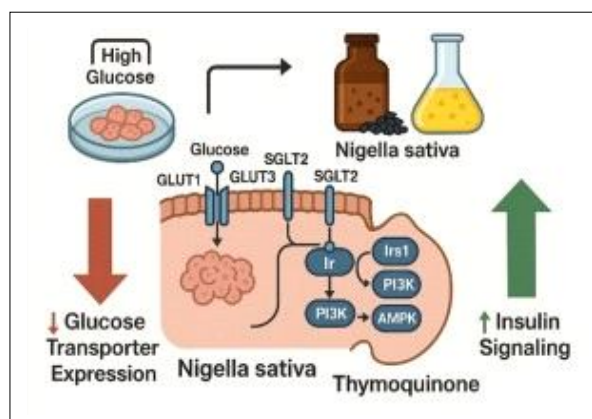


Fig 6: Impact of TQ and NS supplementation on the downstream pathways of glucose metabolism in high-glucose environments.

by TQ and NS, promoting better glycogen metabolism and decreased oxidative stress (Liang *et al.*, 2020; Rayasam *et al.*, 2009; Paeng *et al.*, 2014).

Together, our results show that TQ and NS influence several pathways related to glucose metabolism, such as glucose transporters, Irs1, PI3k/Akt, AMPK and Gsk3 β (Fig 6). Their promise as treatment agents for diabetic nephropathy is highlighted by these effects, which improve insulin sensitivity and reduce hyperglycemia. To validate these protective effects, further extensive *in vivo* and clinical research is necessary.

CONCLUSION

In NRK-52E renal proximal tubular cells, high glucose exposure reduces cell survival, interferes with insulin signaling, and modifies glucose transporter expression. By lowering the expression of Glut1, Glut2, Glut3, and SglT2, increasing Irs1, PI3k/Akt and AMPK signaling, and suppressing Gsk3 β activity, treatment with *Nigella sativa* (NS) extract and thymoquinone (TQ) reduced these effects and promoted glycogen synthesis and glucose utilization.

TQ's potential as a therapeutic agent for avoiding or lessening diabetic nephropathy was highlighted by its better effectiveness than NS in repairing impaired glucose transporter expression and metabolic signaling. These results shed light on the mechanisms behind NS and TQ's renoprotective and antidiabetic actions. To confirm and expand these protective benefits, more *in vivo* and clinical research is necessary.

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Conflict of interest

The authors declares no conflicts of interest to report regarding the present study.

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