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Murrya Koenigii Extract Attenuates Gene Expression of Endothilin-1 and VEGFR-2 and Upregulates NF-KB Genes in NRK-52 Cells Exposed to Glucotoxicity

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KEYWORDS

Murraya koenigii, Endothelin-1, VEGFR-2, HIF-1α genes

ABSTRACT:

Introduction: The Murraya koenigii plant, a member of the Rutaceae family, is renowned for its immunomodulatory and nephroprotective effects. Its leaves contain carbazole compounds known for their revitalizing properties, making Murraya koenigii a significant medicinal plant often utilized in Indian cuisine.

Aim: This study aimed to examine the influence of Murraya koenigii extract on gene expression in NRK-52E cells exposed to glucotoxic conditions.

Materials and methods: Fresh Murraya koenigii plants from Arakkonam, Ranipet District were harvested, washed, and dried. The dried plants were powdered and stored airtight. From this, 50 grams were methanol-extracted via Soxhlet for 4 days, yielding 12 grams of crude extract. A working solution was prepared by dissolving the crude extract powder (20mg) in DMSO (1 ml) for cell line treatment.

Results: The exposure to the extract significantly affected gene expression levels. Specifically, it attenuated the expression of Endothelin-1 and VEGFR-2 genes associated with certain physiological processes. Conversely, the extract led to upregulating HIF-1α, TNF-α, and NF-κB genes, indicating potential modulation of cellular responses and signaling pathways.

Conclusion: Murraya koenigii extract may offer renoprotective benefits against glucotoxicity-induced cellular damage by acting on Endothelin-1, VEGFR-2, and HIF-1α genes, while its immunomodulatory activity may involve upregulation of TNF-α and NF-κB genes.

1. Introduction

Murraya koenigii, an esteemed member of the Rutaceae family, holds a paramount position in traditional medicine. Renowned for its multifaceted benefits, the leaves of Murraya koenigii are rich in carbazole compounds, known for their revitalizing properties and integral role in Indian culinary practices. Alongside carbazoles, these leaves harbor a diverse array of compounds such as terpenoids, flavonoids, and phenolics, each contributing to its medicinal prowess. These bioactive constituents have been extensively

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studied and have shown promising therapeutic effects, including antioxidative, immunomodulatory, neuroprotective, hepatoprotective, antidiabetic, antitumor, and anti-inflammatory activities. Notably, in various animal models, Murraya koenigii extracts have exhibited nephroprotective properties, further underscoring its potential as a valuable botanical resource for holistic health and well-being.[1-7] Furthermore, Murraya leaves are employed in the management of diarrhea and dysentery, attributed to their potent antifungal and antimicrobial properties.[8-10] Murraya koenigii juice is used to manage renal pains.[11] koenigii paste with milk is used to cure curing poisonous bites.[12]

2. Aim

This study aims to investigate the gene expression patterns of NRK-52 cells in cell culture exposed to high glucose levels and various concentrations of Murraya koenigii extract.

3. Methods

Murraya koenigii extract preparation

Fresh and healthy Murraya koenigii plants were harvested from an agricultural field in Arakkonam, Ranipet District. After washing, 500 grams of fresh plants were shade-dried for 3 days at room temperature. The dried sample was powdered using a Venus blender (India) and stored in a sterile, airtight container. Fifty grams of the powdered sample were then extracted with methanol (99%) using a Soxhlet apparatus for 4 days. The resulting extract was concentrated to dryness using a rotary evaporator, yielding 12 grams of crude extract. A working solution was prepared by dissolving the crude extract powder (20mg) in DMSO (1ml), which was then used to treat the cell lines with varying concentrations of the plant sample.

Cell culture

Table 1 Cell Culture Protocol	
Cells cultured	NRK-52E
Culture medium	DMEM
Supplements	Fetal bovine serum 10% (FBS, heat-inactivated), Penicillin 100 µg/mL, 100 µg/mL amphotericin, Streptomycin 100 µg/mL, Sodium bicarbonate 3.7 g/L
Environment	37°C in an environment with 5% CO2
Cells Harvested	Confluence reached - 85 to 90% Cells seeded after Trypsin treatment- 1x 10^6 Cell treatment time with plant extract- 24 hours

Table 2- Groups Analysed	
1.	1.Low glucose 5.5mM (1g/L)
2.	2.High glucose 30Mm (4.5g/L)
3.	3.High glucose 30Mm(4.5g/L) + 50µg/ml extract
4.	4.High glucose 30Mm(4.5g/L) + 100µg/ml extract
5.	5.High glucose 30Mm(4.5g/L) + 200µg/ml extract
6.	6.High glucose 30Mm (4.5g/L)+ 300µg/ml extract
7.	7.High glucose 30 Mm (4.5g/L) +400 µg/ml extract

MTT assay

The cell viability was assessed using the MTT assay from Sigma Aldrich. NRK-52 cells were cultured with different concentrations of Murraya extract in 96-well plates and incubated for 24 hours \ at 37°C in a 5% CO2 environment. Following a 4-hour incubation in darkness at 37°C and DMSO treatment, absorbance was measured at 570 nm to determine % cell viability, according to the protocol.[12]

RNA Isolation and c- DNA library construction

RNA isolation was done using TRIZOL reagent by standard lab protocol involving precipitation by isopropanol and centrifugation at 12,000rpm at 4°C for 20 minutes.[13] RNA quantification was done by nanodrop.

Real-time PCR analysis

The real-time qPCR was conducted for SIRT1, HIF-1α, ET-1, and VEGFR-2 using SYBR green qPCR Mastermix (Takyon, Eurogentec). (Forward: F and Reverse: R) details are shown in Table 3.

Journal of Chemical Health Risks

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Table 3- Primer sequences		
Gene	**Primer**	**Sequence**
TNF-α	F	5′-CTTCTCCTTCCTGATCGTGG-3′
	R	5′-GCTGGTTATCTCTCAGCTCCA-3′
TGF-β	F	5′-TCGCCAGAGTGGTTATCTT-3′
	R	5′-TAGTGAACCCGTTGATGTCC-3′
NF-κB	F	5′-CCATGACAGCAAATCTCC-3′
	R	5′-TAAACTTCATCTCCACCCC-3′
HIF-1α:	F	5′GCCGCTGGAGACACAATCAT 3′
	R	5′GAAGTGGCTTTGGCGTTTCA 3′
ET-1:	F	5′CAGGGCTGAAGACATTATGG AGA3′
	R	5′CATGGTCTCCGACCTGGTTT3′
VEGFR-2:	F	5′CGGACAGTGGTATGGTTCTTG C3′
	R	5′GTGGTGTCTGTGTCATCGGAG TG3′

The following thermal cycler program were performed for PCR amplification. 3 minutes at 95 °C for initial denaturation, denaturation at 95°C for 10 seconds, and 60°C for primer annealing, the cycle threshold (CT) values obtained were analyzed by the 2 – ΔΔCT formula.

4. Results

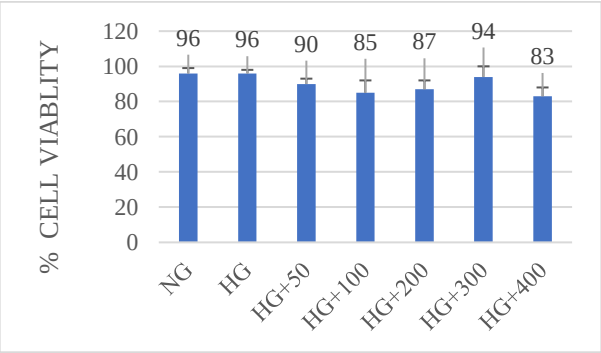

Fig 1: Viability of NRK-52 Cells MTT Assay.

Normal glucose (NG) treated cells, High glucose (HG) treated cells, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400µg concentration Murraya koenigii extract treatment for the Viability of NRK-52 Cells which showed in Figure 1.

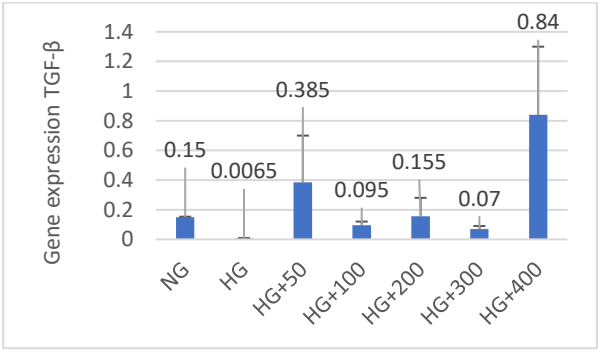

Fig 2: Relative gene expression of TGF-β gene

NG, HG, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400 µg concentration Murraya koenigii extract treatment for the gene expression of β gene were shown in Figure 2.

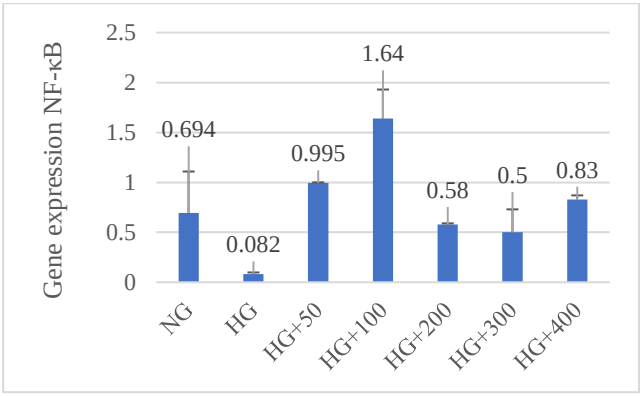

Fig 3: Relative gene expression of NF-κB gene

NG, HG, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400 µg concentration Murraya koenigii extract treatment for NF-κB gene expression shown in Figure 3.

Journal of Chemical Health Risks

www.jchr.org

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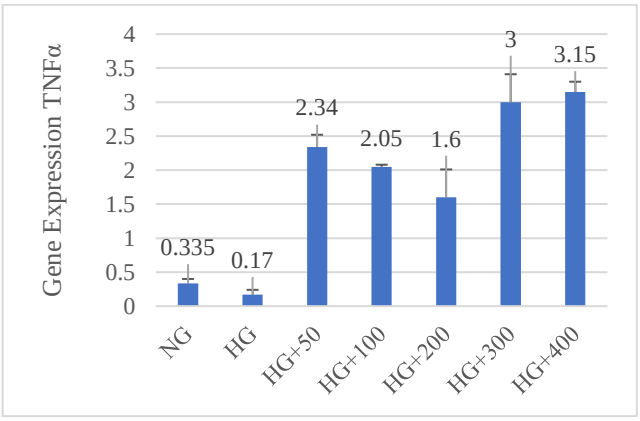

Fig 4: Relative gene expression of TNF-α gene

NG, HG, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400 µg concentration Murraya koenigii extract treatment for the TNF-α gene were shown in Figure 4.

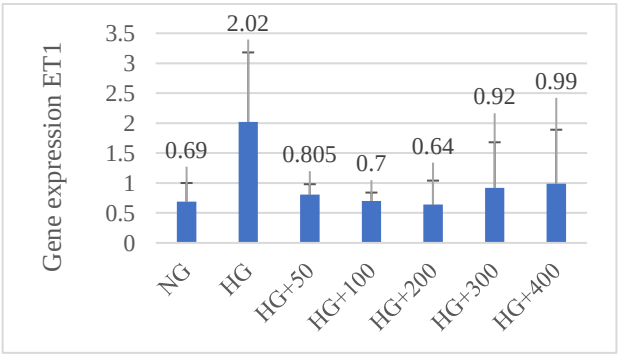

Fig 5: Relative gene expression of ET-1 gene

NG, HG, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400 µg concentration Murraya koenigii extract treatment for ET-1 gene expression shown in Figure 5.

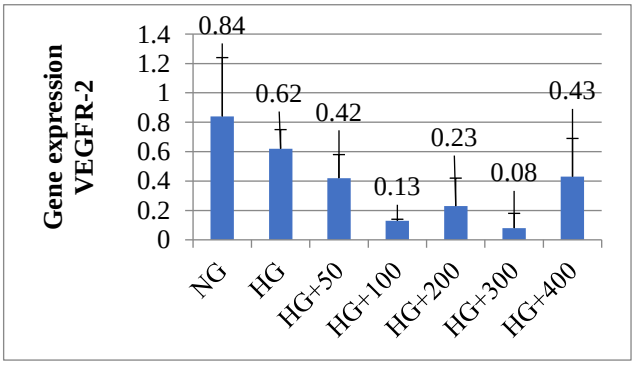

Fig 6: Relative gene expression of VEGFR-2 gene

NG, HG, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400 µg concentration Murraya koenigii extract treatment for the expression of VEGFR-2 gene were shown in Figure 6.

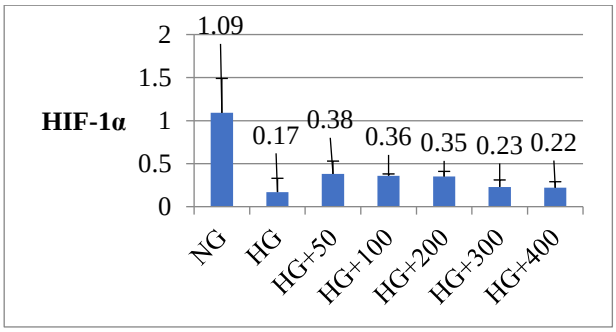

Fig 7: Relative gene expression of HIF-1α gene

NG, HG, HG+50,100,200,300,400- High glucose exposed cells treated with 50,100,200,300,400 µg concentration Murraya koenigii extract treatment for the gene expression of HIF-1α shown in figure 7.

Exposure of renal tubular epithelial cells NRK-52 to high glucose (30 mM) and Murraya extracts of various concentrations did not significantly change cell viability. Murraya extract exposure increased the expression of TGF-β in cells predominantly in 50mcg, 200mcg, and 400mcg concentrations. Murraya extract exposure increased the expression of TNF-α in all concentrations in a dose-related manner. Murraya extract exposure increased expression of NFKB in all concentrations predominantly in 50mcg and 100mcg concentrations. Murraya extract caused a dose-related reduction in the expression of ET1 genes from the 50 to 200 mcg dose range and VEGFR2 expression from the 50 to 300 mcg dose range. These two genes were upregulated by high glucose exposure.

5. Discussion

Our study shows that Murraya extract stimulates the expression of important immune genes like NFKB, TNF-α, and TGF-β. Murraya extract also reduces the expression of ET-1 and VEGFR2. The immune-stimulating properties may be detrimental in the case of diabetic nephropathy. In a model involving immunity to ovalbumin, Murraya extract was shown to increase NO production and phagocytic activity and stimulate humoral immunity. (Shah.A.S 2008).[13] Our studies show upregulate inTGF-β, NFKB, and TNF-α genes which

Journal of Chemical Health Risks

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also confirms the immune stimulatory potential of Murraya.

Murraya has been shown to have a nephroprotective effect in rat diabetic nephropathy models and it was demonstrated to be due to antioxidant capacity and renal myeloperoxidase activity (Yankuzo. H 2011).[14] Murraya extract helped to maintain high glutathione (GSH) and superoxide dismutase (SOD) levels in kidneys and reduced cyclophosphamide-induced nephrotoxicity (Mahipal 2017)[15] Murraya was also found to decrease serum creatinine and help in maintaining normal renal histology and help tissue regeneration in kidneys(.Purnuru 2014).[16] Though all these activities were previously attributed to antioxidant properties, the decrease in ET-1 gene and VEGFR-2 gene expression following Murraya extract treatment in renal tubular cells may contribute to the nephroprotective activity of Murraya koengii. There is enough literature regarding the usefulness of antagonizing ET-1, and VEGFR-2 in treating diabetic nephropathy. Plasma and urinary Endothelin-1 levels were elevated in patients with diabetic nephropathy and antagonizing ET1 receptors was shown to be beneficial to diabetic kidneys (Peppa-Patrikiou et al., 1998; Wenzelet et al., 1999).[17,18] A VEGFR2 pathway-activation blockade improved renal function and alleviated glomerular damage in diabetic mice models (Lavoz et al., 2020).[19] The increase in HIF-1α gene expression following Murraya extract treatment in renal tubular cells may also contribute to the nephroprotective activity. Yu. L *et al* have shown that HIF-1α activated Parkin/PINK1-mediated mitophagy and this action prevented apoptosis and ROS production in HK-2 cells subjected to high glucose exposure thereby alleviating Diabetic nephropathy.[20]

6. Conclusion:

Our study demonstrates that Murraya koenigii extract can modulate the gene expression pattern in NRK-52 cells under high glucose conditions, suggesting its potential immunomodulatory and nephroprotective effects.

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