Effect of *Borreria hispida* Extract on SIRT1, HIF-1α, ET-1 and VEGFR-2 Gene Expression in NRK-52E Cells Subjected to Glucotoxicity

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**Abstract:** This study aimed to investigate the impact of *Borreria hispida* extract on gene expression in NRK-52E cells under conditions of glucotoxicity. Gene expression analysis was conducted using RT-PCR following the exposure of cultured NRK-52E cells to glucotoxic conditions and varying concentrations of *Borreria hispida* extract. The results demonstrated a dose-dependent increase in SIRT1 gene expression and a concomitant decrease in HIF-1α, ET-1, and VEGFR-2 gene expressions upon treatment with *Borreria hispida* extract. Additionally, molecular docking studies suggested the potential inhibition of Rho-kinase as a mechanistic explanation for these effects. *Borreria hispida* extract may confer renoprotective benefits against glucotoxicity-induced cellular damage. The potential therapeutic utility of *Borreria hispida* extracts in managing renal complications associated with conditions such as diabetes. Furthermore, our docking studies shed light on the potential molecular mechanisms underlying the observed effects, suggesting interactions between phytochemicals present in *Borreria hispida* extract and the JNK-1 protein. These interactions may contribute to the augmentation of SIRT1 activity, further bolstering the extract’s therapeutic utility in DKD.

**Introduction**

Diabetic kidney disease (DKD) affects about one in three people with diabetes. By 2035, about half of the world population will be affected by diabetes (Sur et al., 2023; Biswas et al., 2023; Roy et al., 2023; Medhi et al., 2023; Biswas et al., 2023), of which a sizeable proportion will develop diabetic kidney disease (Andersen et al., 1983; Price et al., 2012; Hallows et al., 2012). Sirtuin 1
(SIRT1) is an important protein encoded by the gene of the same name that takes part in the metabolism of glucose and fat in the body (Moynihan et al., 2005; Purushotham et al., 2009; Chuang et al., 2011; Hasegawa et al., 2013; Chakraborty et al., 2021; Acharya et al., 2023; Sarkar et al., 2023; Pawar et al., 2023). In various in vivo and in vitro diabetic nephropathy models, reduced SIRT1 gene expression in diabetic kidney tissues was correlated with the severity of proteinuria (Susztak et al., 2006; Nihalani and Susztak, 2013), and an increase in SIRT1 was associated with improvement in renal lesions (Hasegawa, 2013). Hyperglycemia decreases SIRT1 expression and increases podocyte apoptosis (Susztak et al., 2006; Yoshino et al., 2011). Tubular SIRT1 decreases renal damage by increasing podocyte nicotinamide mononucleotide (NMN) concentration (Thomas et al., 2004; Kume et al., 2007; Zhuo et al., 2011). SIRT1 attenuates TGF-β1-induced mesangial cell apoptosis (Chiang et al., 2019). SIRT1 prevents mesangial proliferation by downregulating the AMPK-mTOR signaling pathway (Song et al., 2022). Hyperglycemia and microangiopathy create a chronic hypoxic state in diabetic kidneys, resulting in an increase in hypoxia-inducible factor-1α (HIF-1α), which in turn can hasten apoptosis via heme oxygenase-1 (HO-1) (Sun et al., 2012; Lange et al., 2010; Issoe et al., 2010; Yu et al., 2022).

High levels of HIF-1α expression are seen in lesions in the kidneys of diabetic animal models (Wenzel et al., 1999) and can promote renal fibrosis-stimulating genes like connective tissue growth factor and plasminogen activator inhibitor 1. Conversely, Yu et al. (2022) 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 (Peppa-Patrikiou et al., 1998). Plasma and urinary endothelin-1 levels were found to be elevated in patients with diabetic nephropathy (Lavoz et al., 2020) and correlate with renal damage and albuminuria. ET receptor antagonists are shown to have anti-proteinuric, anti-inflammatory and anti-proliferative properties and are under study. An activated VEGFR2 pathway leads to kidney damage in diabetic patients. A murine model showed that the blockade of VEGFR2 kinase alleviated kidney damage due to diabetes. In this experiment, we intend to study the effect of *Borreria hispida* extract on the gene expression of SIRT1, endothelin-1, VEGFR-2, and HIF-1 alpha in NRK-52E (rat renal proximal tubular epithelial cell) cells subjected to glucotoxicity.

### Materials and Methods

#### Plant collection and preparation of crude extract

Fresh and healthy plants of *Borreria hispida* were collected from the wild in Arakkonam, Ranipet District. Five hundred grams of fresh plants were washed and shade-dried for 3 days at room temperature. The dried sample was made into a fine powder using a blender (Venus Blender, India) and the powdered sample was stored in a sterile, airtight container until further use. Fifty grams (50g) of the powdered sample were packed, placed in a soxhlet apparatus, and extracted with methanol (99%) for 3 days. The extract obtained was concentrated to dryness with the help of a rotary evaporator. A total quantity of 5.0 g of crude extract was obtained after dryness. The working solution was prepared by dissolving 20 mg of crude extract powder in 1 ml of dimethyl sulfoxide (DMSO), and the cell lines were treated with different concentrations of the plant sample.

#### Cell line and maintenance

NRK-52E cells, derived from rat renal proximal tubular epithelial cells and obtained from the National Centre for Cell Science in Pune, India, were cultured in Dulbecco’s modified Eagle’s medium (DMEM). The culture medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS), along with 100 μg/mL penicillin, 100 μg/mL streptomycin, 100 μg/mL amphotericin and 3.7 g/L sodium bicarbonate. Cells were maintained in a controlled environment at 37°C with 5% CO2. Upon reaching a confluence of 85 to 90%, cells were harvested, with approximately 1x10⁶ cells seeded after trypsin treatment. For the cell treatment with the plant extract, a duration of 24 hours was employed to allow for sufficient exposure to the extract.

#### NRK 52E Cell Treatment with Varying *Borreria hispida* Extract Concentrations

The experimental conditions consisted of seven distinct groups. The first group was maintained under low glucose conditions with a concentration of 5.5 mM (1 g/L). The second group was exposed to high glucose levels, with a concentration of 30 mM (4.5 g/L). Subsequent experimental groups were subjected to high glucose conditions (30 mM or 4.5 g/L) supplemented with varying concentrations of the extract: 50 μg/ml (third group), 100 μg/ml (fourth group), 200 μg/ml (fifth group), 300 μg/ml (sixth group), and 400 μg/ml (seventh group). These experimental setups allowed for the assessment of the effects of the extract at different concentrations on cells cultured under normal and high-
glucose conditions. Such an approach facilitated the exploration of potential dose-dependent responses and provided valuable insights into the extract's efficacy in mitigating the adverse effects of glucotoxicity.

**RNA isolation**

RNA isolation was done from cells using TRIZOL reagent by standard lab protocol involving centrifugation \((12,000\times g)\) for 20 minutes at 4°C and precipitation by isopropanol (Simms et al., 1993). Nano drops did RNA quantification. The cDNA library was constructed with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

**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).

**SIRT1:**

Forward Primer: 5’TGTGGTAGAGCTTGCATTGATCTT 3’
Reverse Primer: 5’GGCCTGTTGCTCTCCTCATT 3’

**HIF-1α:**

Forward Primer: 5’GCCGCTGGAGACACAATCAT3’
Reverse Primer: 5’GAAGTGGCTTTGGCGTTTCA 3’

**ET-1:**

Forward Primer: 5’CAGGGCTGAAGACATTATGGAGA3’
Reverse Primer: 5’CATGGTCTCCGACCTGGTTT3’

**VEGFR-2:**

Forward Primer: 5’CGGACAGTGTATGGTTCTTGC3’, Reverse Primer: 5’GTGGTGCTGTCATCGGAAGTG3’

The PCR amplification was performed with the following thermal cycler program: Initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 10 seconds, primer annealing at 60°C for 20 seconds, and extension at 72°C for 1 minute. The cycle threshold (CT) values obtained were analyzed by the \(2^{-\Delta\Delta CT}\) formula.

**Docking study**

A comprehensive approach involving several key components was adopted for the docking protocol.

Ligands, totalling 15 in number, were sourced from the PubChem database. These ligands constitute the molecules under investigation for their interactions with the target protein. The protein structure utilized in the docking studies was that of JNK-1, with the PDB code 2NO3, retrieved from the RCSB Protein Data Bank (Altunkaya et al., 2016). AutoDockVina, a widely utilized molecular docking software, was employed for the docking simulations (Trott and Olson 2009). The docking experiments were executed on the PyRx platform, facilitating efficient setup and management of the docking runs. To visualize the hydrogen bond interactions between the ligands and the amino acid residues of the protein, the PYMOL software was employed (DeLano, 2002). This integrated docking protocol enabled the systematic exploration of ligand-protein interactions, offering valuable insights into potential binding modes and affinities, thus contributing to understanding ligand-target interactions at the molecular level.
Relative gene expression of SIRT 1

Upon exposure to high glucose conditions, the relative expression of the SIRT1 gene was 0.1362 ± 0.051 as compared to normal glucose conditions (1.73±1.44). Treatment with various concentrations of Borreria hispida extract counteracted the decrease in SIRT1 expression in a dose-related manner, starting from 50 mcg (4.026±1.81) to the highest relative expression at 300 mcg.

![Figure 2. Relative gene expression of Endothelin-1 (NG-Normal Glucose treated cells; HG-High Glucose Treated Cells; HG+ 50, 100, 200, 300, 400; High glucose exposed cells treated with 50, 100, 200, 300, 400µg/ml concentration of Borreria hispida extract).](image)

![Figure 3. Relative gene expression of HIF-1α (NG-Normal Glucose treated cells; HG-High Glucose Treated Cells; HG+ 50, 100, 200, 300, 400; High glucose exposed cells treated with 50, 100, 200, 300, 400µg/ml concentration of Borreria hispida extract).](image)
(16.17±6.34). At 400 mcg, the therapeutic benefit decreased to 3.69±2.8, which was still higher than the gene expression at glucotoxic levels and normoglycemia.

**Relative gene expression of endothelin-1**

Upon exposure to high glucose conditions, the relative gene expression of ET-1 increased to 3.06±2.10 as compared to the normoglycemic condition (1.98±1.71). Treatment of cells with various concentrations of *Borreria* extracts counteracted the increase in ET-1 expression from 1.75±1.30 at 50 μg/ml to 1.39±0.93 at 300 μg/ml. The lowest gene expression point was 300 μg/mL in treated cells. At 400 μg/ml, the therapeutic benefit decreased, and again, gene expression increased above normoglycemic levels to 2.55±2.96.

**Relative gene expression of HIF-1α**

The relative expression of HIF-1α is 0.22±0.13, 0.30±0.39, 0.23±0.17, and 0.47±0.40, respectively, in high glucose-exposed cells treated with 50, 100, 200, and 300 μg/ml concentrations of *Borreria hispida* extract. The lowest point of gene expression was at 200 μg/mL concentration in treated cells. At 400 μg/ml, the gene expression increased to levels (0.82±0.66). Fifteen phytochemical ligands of *Borreria hispida* were identified and docked from a literature search.

**Relative gene expression of VEGFR2**

Upon exposure to high glucose conditions, the relative gene expression of ET-1 increased to 3.87±1.90 as compared to the normoglycemic condition (1.09±0.45). The extract counteracted the increase. The relative expression of VEGFR2 is 0.95±0.56, 2.38±1.65, 1.49±0.12, 0.29±0.11, and 1.09±0.16, respectively, in high glucose-exposed cells treated with 50, 100, 200, 300, and 400 μg/mL concentration extract, respectively.

**Docking Study for JNK1 and SIRT1**

JNK1 phosphorylated human SIRT1 on three sites: Ser27, Ser47 and Thr530. Ubiquitination occurs in the SIRT1 protein after phosphorylation, followed by degradation by the proteasome. In *vivo*, SIRT1 undergoes extensive degradation in hepatocytes in obesity as a consequence of persistent activation of JNK1. The degradation leads to inhibition of SIRT1 function, which contributes to developing hepatic steatosis. Hence, we did a docking study of the phytochemicals of *Borreria hispida* for the JNK1 protein structure to elicit whether phytochemicals could bind and influence JNK1 and, thereby, SIRT1 activity. Since *Borreria* extract showed good SIRT-1-enhancing action, we studied the docking properties of the phytochemicals of *Borreria hispida* on the JNK-1 protein, which has an effect on the SIRT-1 level. Fifteen phytochemical ligands of *Borreria hispida* were identified and docked from a literature search.

**Discussion**

Our study has shown that *Borreria hispida* extract has a positive effect on the gene expression of SIRT1 and a negative effect on the gene expression of HIF-1α, ET-1, and VEGFR-2 in vitro. These properties, if present in...
Table 1. Docking study.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Ligands</th>
<th>Docking Score</th>
<th>Amino Acids</th>
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<tbody>
<tr>
<td>1</td>
<td>Ligand 859</td>
<td>-7.1</td>
<td>Asp169</td>
</tr>
<tr>
<td>2</td>
<td>Borreline</td>
<td>-7.4</td>
<td>Asp151, Asp169, Lys153</td>
</tr>
<tr>
<td>3</td>
<td>Dalspinin</td>
<td>-7.0</td>
<td>Lys153, Gln37, Asn 114</td>
</tr>
<tr>
<td>4</td>
<td>DeacetylAsperulosidic acid</td>
<td>-7.7</td>
<td>Thr188, Lys153</td>
</tr>
<tr>
<td>5</td>
<td>Epigallocatechin</td>
<td>-6.7</td>
<td>Ala36</td>
</tr>
<tr>
<td>6</td>
<td>Gallic acid</td>
<td>-5.8</td>
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</tr>
<tr>
<td>7</td>
<td>Hesperitin</td>
<td>-6.8</td>
<td>Asp151, Asn114, Gln37</td>
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<tr>
<td>8</td>
<td>Isorhamnetin</td>
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<tr>
<td>9</td>
<td>Kaempferol</td>
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<td>Asn114, Gln37</td>
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<tr>
<td>10</td>
<td>Quercetin</td>
<td>-6.8</td>
<td>Asp151, Arg69</td>
</tr>
</tbody>
</table>

Figure 5. JNK-1 structure (2NO3) containing phytochemical Borreline in active 859 native ligand binding site.

Figure 6. JNK-1 structure (2NO3) containing phytochemical Dalspinin in active 859 native ligand binding site.
vivo, will also tremendously benefit from reversing pathologic changes due to diabetic nephropathy. Further literature searches have shown that quercetin, kaempferol, isorhamnetin, kaempferol, epigallocatechin, and spermatin, which are present in Borreria hispida, have SIRT1-increasing properties in cardiomyocytes, hepatocytes, and immune cells. Further quercetin and isorhamnetin, kaempferol, and speretin compounds were shown to have JNK-1-inhibiting properties in cardiomyocytes, neuronal cells, and various other models, as follows: Huang, Liqing, et al. (2016) have shown that the protective effects of isorhamnetin on rat cardiomyocytes against anoxia and reoxygenation-induced injury are mediated by SIRT1. Isorhamnetin inhibited the phosphorylation of ERK, JNK, IkBa, and NF-κB (p65) activated by LPS in vivo by affecting the signaling pathways of MAPK and NF-κB and alleviated neutrophil infiltration and edema in the ALI model (Qi et al., 2018). De Boer et al. (2006) have shown that quercetin upregulates SIRT1 and helps reverse aging. Quercetin has a SIRT1-dependent immunomodulatory effect van Horssen et al. (2008) and Wang et al. (2018) have shown that quercetin reverses NF-kB-mediated inflammation induced by LPS in lung fibroblasts.

Guo Zhen et al. (2015) have shown that kaempferol protects rat cardiac cells against anoxia via SIRT1 upregulation. Shokri Afra et al. (2019) have shown that speretin is a potent bio-activator that activates the SIRT1-AMPK signaling pathway in HepG2 cells. Hesperetin rescued LPS-induced neuronal apoptosis by reducing the expression of phosphorylated c-Jun N-terminal kinases (Muhammad et al., 2019). In this experiment, we have shown that Borreria hispida extract can elevate SIRT1 gene expression in renal tubular cells exposed to glucotoxicity, which may confer good therapeutic benefits in diabetic nephropathy.

Further docking studies have shown that Borreline, Dalspinin, and Deacetylasperulosidic Acid also had a higher docking score to the JNK-1 structure than quercetin and isorhamnetin, and these chemicals may also synergistically contribute to SIRT1 augmentation via the JNK1 pathway. Hence, we can infer that Borreria hispida has various phytochemicals that may act synergistically to counteract the effects of hyperglycemia on kidneys, and SIRT1 stimulation demonstrated in cardiomyocytes, liver cells, and inflammatory models may hold good in renal cells as well. Further, Borreline and Dalspinin present in Borreria may also possess similar activity, which needs to be confirmed further. Borreria hispida extract further negatively affects HIF-1α, ET-1, and VEGFR-2 genes, which may provide added benefit in cases of diabetic kidney disease.

Conclusion

In conclusion, our study illuminates the potential therapeutic efficacy of Borreria hispida extract in mitigating the adverse effects of glucotoxicity on renal cells, as evidenced by its modulation of gene expression profiles in NRK-52E cells. Notably, treatment with Borreria hispida extract led to a dose-dependent increase in SIRT1 gene expression while concurrently attenuating the expression of HIF-1α, ET-1, and VEGFR-2 genes under conditions of glucotoxicity. These findings hold significant implications for the management of diabetic kidney disease (DKD), a condition that poses a substantial burden on global healthcare systems. By targeting key genes implicated in the pathogenesis of DKD, Borreria hispida extract demonstrates promising
renoprotective potential. Of particular relevance is the observed elevation of SIRT1 expression, a protein known for its crucial role in glucose and fat metabolism and its protective effects against renal damage in various disease models. Furthermore, our docking studies shed light on the potential molecular mechanisms underlying the observed effects, suggesting interactions between phytochemicals present in Borreria hispida extract and the JNK-1 protein. These interactions may contribute to the augmentation of SIRT1 activity, further bolstering the extract's therapeutic utility in DKD. Overall, our findings contribute to a deeper understanding of the pharmacological properties of Borreria hispida extract and its potential application in the management of renal complications associated with diabetes. Future studies exploring the in vivo effects of the extract, as well as elucidating the specific mechanisms of action underlying its observed effects, will be essential for advancing its clinical translation and therapeutic utility in DKD management.

Conflict of Interest
The authors declare no conflict of interest.

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