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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-1λ, 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

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(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; Chakrovorty 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-b1-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; Feng et al., 2021; Isoe et al., 2010; Yu et al., 2022).

High levels of HIF-1 α 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 $1x10^6$ 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-

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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×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′CGGACAGTGGTATGGTTCTTGC3′, Reverse Primer;5′GTGGTGTCTGTGTCATCGGAGTG3′

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.

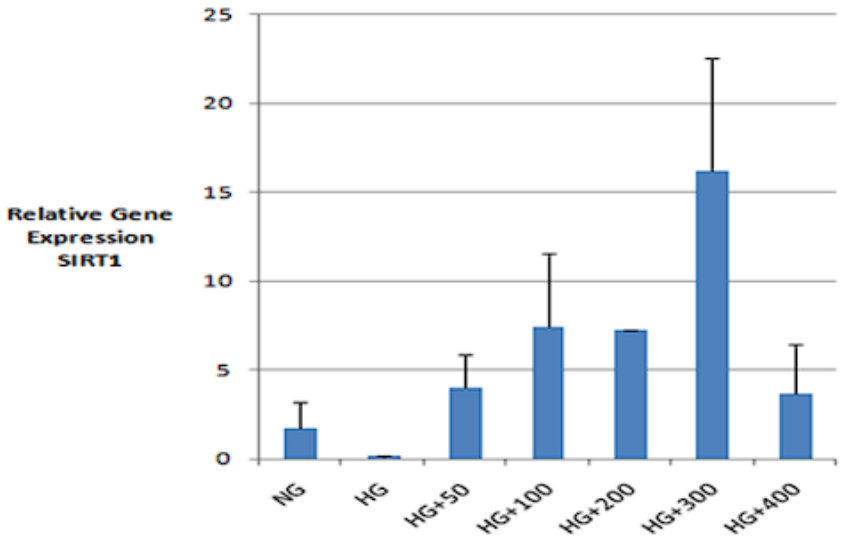

Figure 1. Relative gene expression of SIRT1 (NG-Normal Glucose treated cells; HG-High Gulucose Treated Cells; HG+ 50, 100, 200, 300, 400; High glucose exposed cells treated with 50, 100, 200, 300, 400µg/ml concentration of *Borreria hispida* extract).

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). Trea-tment with various concentrations of Borreriahispida 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

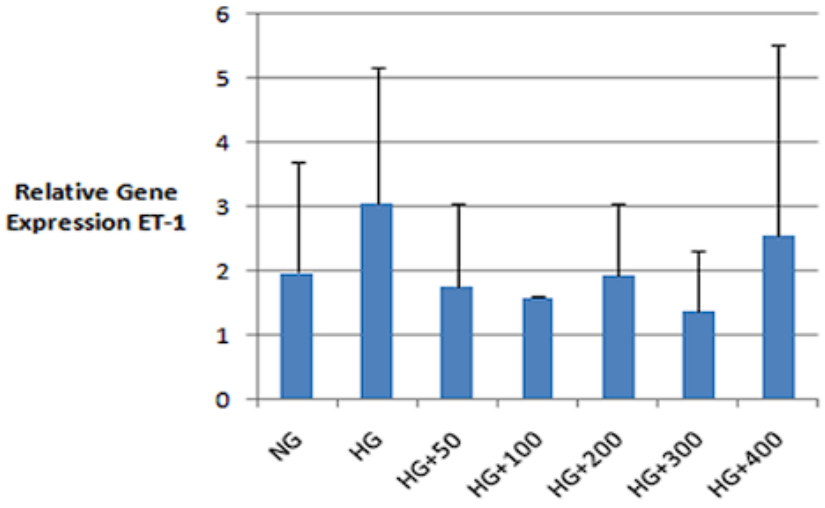

Figure 2. Relative gene expression of Endothelin-1 (NG-Normal Glucose treated cells; HG-High Gulucose Treated Cells; HG+ 50, 100, 200, 300, 400; High glucose exposed cells treated with 50, 100, 200, 300, 400µg/ml concentration of *Borreria hispida* extract).

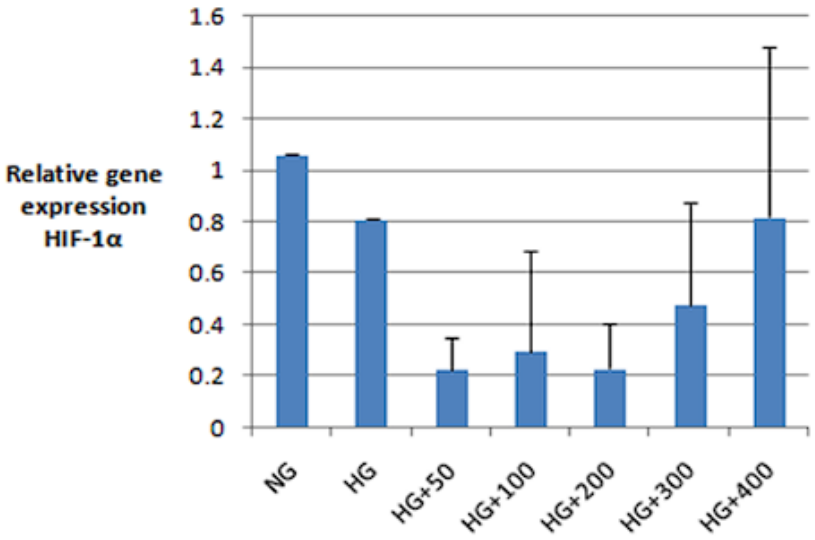

Figure 3. Relative gene expression of HIF-1*α* (NG-Normal Glucose treated cells; HG-High Gulucose Treated Cells; HG+ 50, 100, 200, 300, 400; High glucose exposed cells treated with 50, 100, 200, 300, 400µg/ml concentration of *Borreria hispida* extract).

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(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.

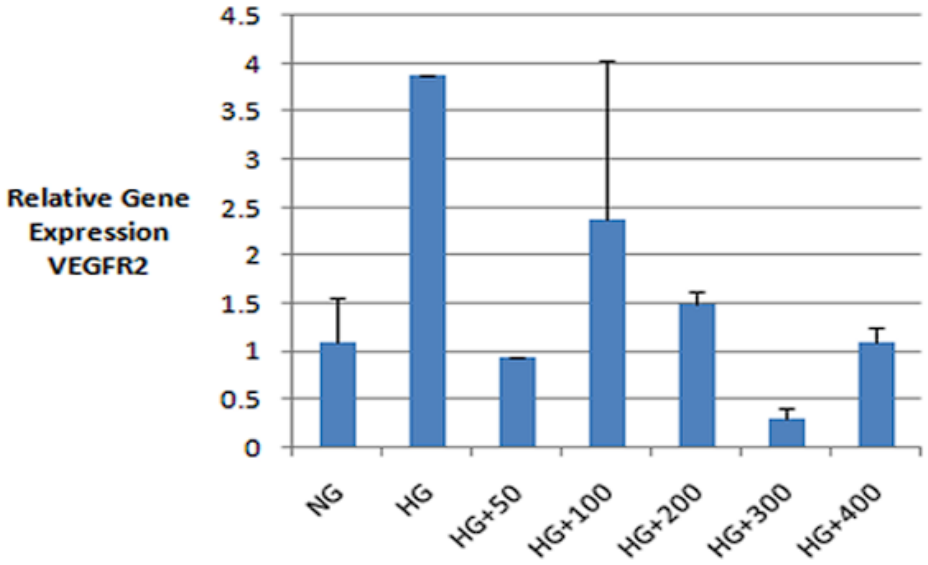

Figure 4. Relative gene expression of VEGFR2 (NG-Normal Glucose treated cells; HG-High Gulucose Treated Cells; HG+ 50, 100, 200, 300, 400; High glucose exposed cells treated with 50, 100, 200, 300, 400µg/ml concentration of *Borreria hispida* extract).

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 the treatment concentrations of 50–300 µg/mL, the gene expression was lower than in normoglycemic-exposed cells (1.06±0.33) and hyperglycemic-non-treated cells (0.81±0.66). At 400 µg/ml concentration, *Borreria hispida* extract-treated cells gene expression increased to levels (0.82±0.66).

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*

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Table 1. Docking study.

Sl. No	Ligands	Docking Score	Amino Acids
1	Ligand 859	-7.1	Asp169
2	Borreline	-7.4	Asp151, Asp169, Lys153
3	Dalspinin	-7.0	Lys153, Gln37, Asn 114
4	DeacetylAsperulosidic acid	-7.7	Thr188, Lys153
5	Epigallocatechin	-6.7	Ala36
6	Gallic acid	-5.8	Arg69, His66, Ala-70
7	Hesperitin	-6.8	Asp151, Asn114, Gln37
8	Isorhamnetin	-6.5	Ser155, Lys153, Asp169, Ser34
9	Kaempferol	-6.5	Asn114, Gln37
10	Quercetin	-6.8	Asp151, Arg69

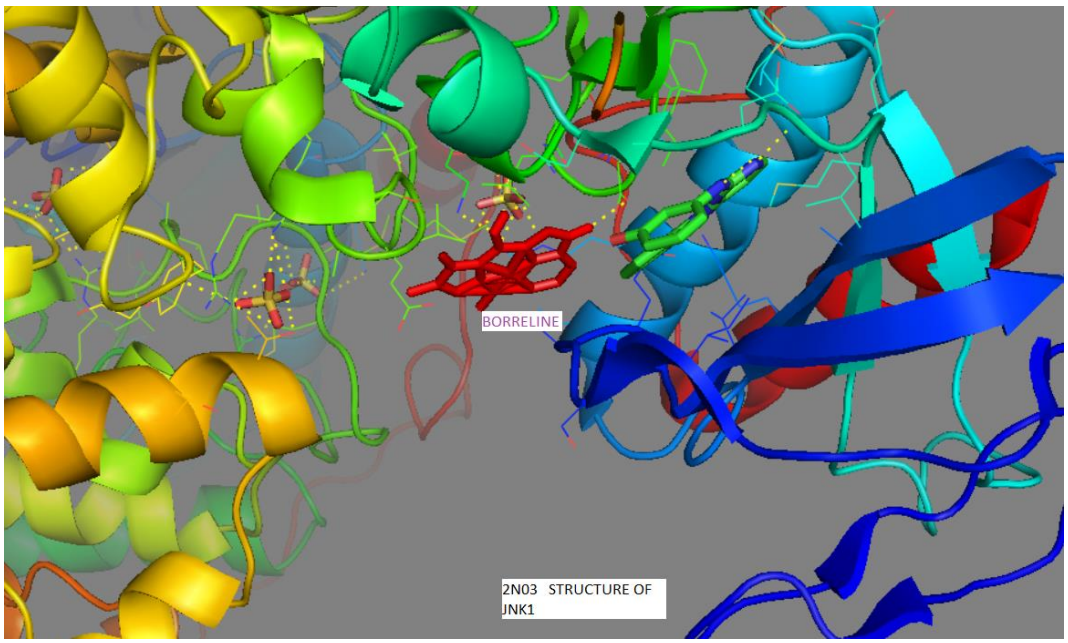

Figure 5. JNK-1 structure ($2NO_3$) containing phytochemical Borreline in active 859 native ligand binding site.

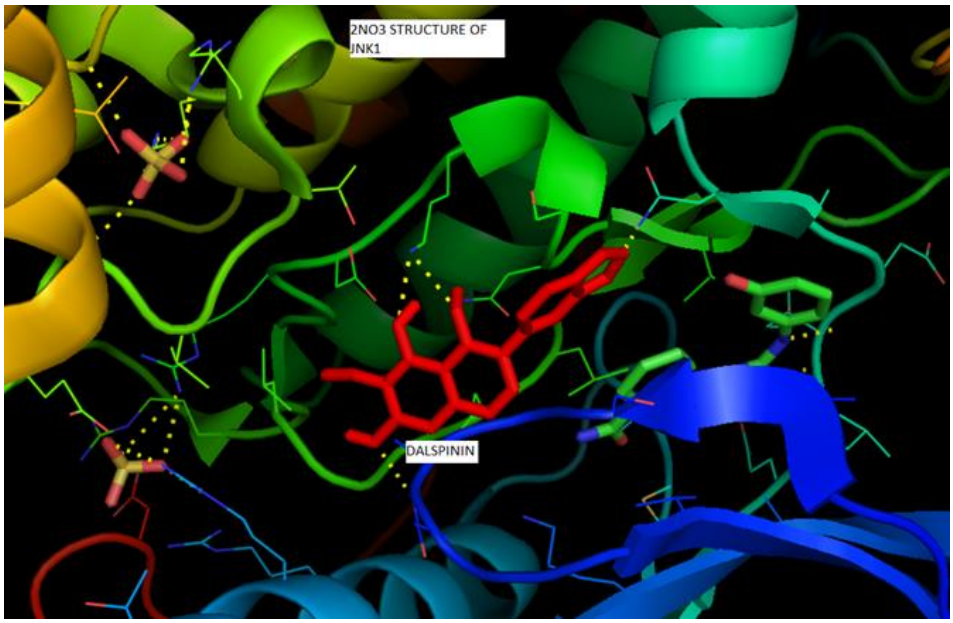

Figure 6. JNK-1 structure ($2NO_3$) containing phytochemical Dalspinin in active 859 native ligand binding site.

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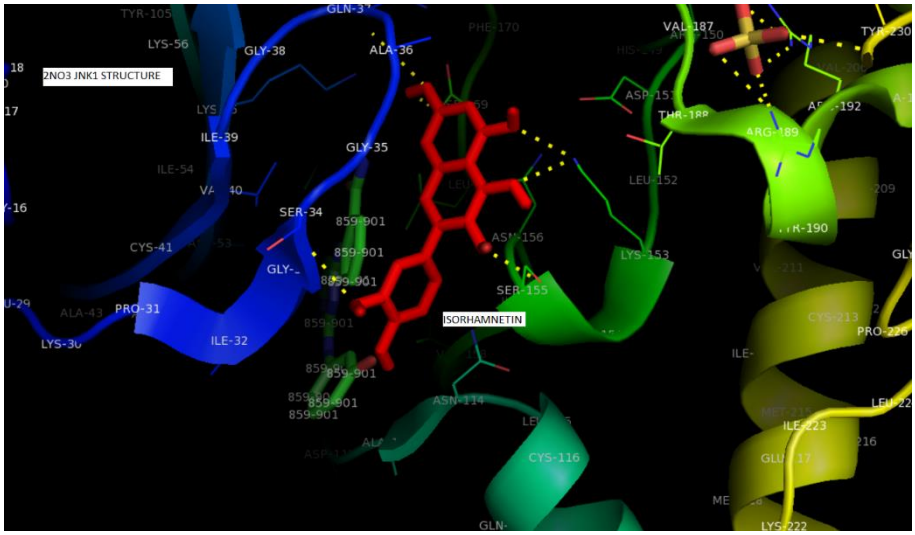

Figure 7. JNK-1 structure ($2NO_3$) containing phytochemical isorhamnetin in an active 859 native ligand binding site.

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, IκBa, 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

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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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References

Acharya, C.K., Das, B., Madhu, N.R., Sau, S., Manna De, M., & Sarkar, B. (2023). A Comprehensive Pharmacological Appraisal of Indian Traditional Medicinal Plants with Anti-diabetic Potential. Springer Nature Singapore Pte Ltd., Advances in Diabetes Research and Management, pp. 163–193, Online ISBN-978-981-19-0027-3. https://doi.org/10.1007/978-981-19-0027-3_8

Altunkaya, A., Bi, C., Bradley, A. R., Rose, P. W., Prli, A., Christie, H., Costanzo, L. Di, Duarte, J. M., Dutta, S., Feng, Z., Green, R. K., Goodsell, D. S., Hudson, B., Kalro, T., Lowe, R., Peisach, E., Randle, C., Rose, A. S., Shao, C., … Burley, S. K. (2016). OUP accepted manuscript. *Nucleic Acids Research*, 1–11. https://doi.org/10.1093/nar/gkw1000

Andersen, A. R., Christiansen, J. S., Andersen, J. K., Kreiner, S., & Deckert, T. (1983). Diabetic nephropathy in type 1 (insulin-dependent) diabetes: An epidemiological study. Diabetologia, 25(6), 496–501. https://doi.org/10.1007/BF00284458

Biswas, T., Behera, B. K., & Madhu, N.R. (2023). Technology in the Management of Type 1 and Type 2 Diabetes Mellitus: Recent Status and Future Prospects. 26 pages, Springer Nature Singapore Pte Ltd., Advances in Diabetes Research and Management. pp. 111–136. Online ISBN-978-981-19-0027-3. https://doi.org/10.1007/978-981-19-0027-3_6

Chakrovorty, A., Bhattacharjee, B., Dey, R., Samadder, A., & Nandi, S. (2021). Graphene: the magic carbon derived biological weapon for human welfare. *Int. J. Exp. Res. Rev.*, 25, 9-17. https://doi.org/10.52756/ijerr.2021.v25.002

Chiang, S. K., Chen, S. E., & Chang, L. C. (2019). A dual role of heme oxygenase-1 in cancer cells. *International Journal of Molecular Sciences*, *20*(1), 1–18. https://doi.org/10.3390/ijms20010039

Chuang, P. Y., Dai, Y., Liu, R., He, H., Kretzler, M., Jim, B., Cohen, C. D., & He, J. C. (2011). Alteration of forkhead box o (foxo4) acetylation mediates apoptosis of podocytes in diabetes mellitus. *PLoS ONE*, *6*(8). https://doi.org/10.1371/journal.pone.0023566

de Boer, V. C. J., de Goffau, M. C., Arts, I. C. W., Hollman, P. C. H., & Keijer, J. (2006). SIRT1 stimulation by polyphenols is affected by their stability and metabolism. *Mechanisms of Ageing and Development*, *127*(7), 618–627. https://doi.org/10.1016/j.mad.2006.02.007

Feng, X., Wang, S., Sun, Z., Dong, H., Yu, H., Huang, M., & Gao, X. (2021). Ferroptosis Enhanced Diabetic Renal Tubular Injury via HIF-1α/HO-1 Pathway in db/db Mice. *Frontiers in Endocrinology*, *12*(February), 1–12. https://doi.org/10.3389/fendo.2021.626390

Gao, Z., Zhang, J., Kheterpal, I., Kennedy, N., Davis, R. J., & Ye, J. (2011). Sirtuin 1 (SIRT1) protein degradation in response to persistent c-Jun N-terminal Kinase 1 (JNK1) activation contributes to hepatic steatosis in obesity. *Journal of Biological Chemistry*, *286*(25), 22227–22234. https://doi.org/10.1074/jbc.M111.228874

Guo, Z., Liao, Z., Huang, L., Liu, D., Yin, D., & He, M. (2015). Kaempferol protects cardiomyocytes against anoxia/reoxygenation injury via mitochondrial pathway mediated by SIRT1. *European Journal of Pharmacology*, *761*, 245–253. https://doi.org/10.1016/j.ejphar.2015.05.056

Hallows, W. C., Yu, W., & Denu, J. M. (2012). Regulation of glycolytic enzyme phosphoglycerate mutase-1 by Sirt1 protein-mediated deacetylation. *Journal of Biological Chemistry*, *287*(6), 3850–3858. https://doi.org/10.1074/jbc.M111.317404

Hasegawa, K., Wakino, S., Simic, P., Sakamaki, Y., Minakuchi, H., Fujimura, K., Hosoya, K., Komatsu, M., Kaneko, Y., Kanda, T., Kubota, E., Tokuyama, H., Hayashi, K., Guarente, L., & Itoh, H. (2013). Renal tubular sirt1 attenuates diabetic albuminuria by epigenetically suppressing claudin-1 overexpression in podocytes. *Nature Medicine*, *19*(11), 1496–1504. https://doi.org/10.1038/nm.3363

Huang, L., He, H., Liu, Z., Liu, D., Yin, D., & He, M. (2016). Protective Effects of Isorhamnetin on Cardiomyocytes Against Anoxia/Reoxygenation-induced Injury Is Mediated by SIRT1. *Journal of Cardiovascular Pharmacology*, *67*(6), 526–537. https://doi.org/10.1097/FJC.0000000000000376

Isoe, T., Makino, Y., Mizumoto, K., Sakagami, H., Fujita, Y., Honjo, J., Takiyama, Y., Itoh, H., & Haneda, M. (2010). High glucose activates HIF-1-mediated signal transduction in glomerular mesangial cells through a carbohydrate response element binding protein. *Kidney International*, *78*(1), 48–59. https://doi.org/10.1038/ki.2010.99

Kume, S., Haneda, M., Kanasaki, K., Sugimoto, T., Araki, S. I., Isshiki, K., Isono, M., Uzu, T., Guarente, L., Kashiwagi, A., & Koya, D. (2007). SIRT1 inhibits transforming growth factor β-induced apoptosis in glomerular mesangial cells via Smad7 deacetylation. *Journal of Biological Chemistry*, *282*(1), 151–158. https://doi.org/10.1074/jbc.M605904200

DeLano, W. (2002). Pymol: An open-source molecular graphics tool. *{CCP4} Newsletter On Protein Crystallography*, *40*, 1–8. http://www.ccp4.ac.uk/newsletters/newsletter40/11_pymol.pdf

Lavoz, C., Rodrigues-diez, R. R., Plaza, A., Carpio, D., Egido, J., Ruiz-ortega, M., & Mezzano, S. (2020). VEGFR2 blockade improves renal damage in an experimental model of type 2 diabetic nephropathy. *Journal of Clinical Medicine*, *9*(2), 1–21. https://doi.org/10.3390/jcm9020302

Medhi, J., Karmakar, M., Barman, A., Mondal, S., & Nag, A. (2023). Diabetic retinopathy stage detection using convolutional fine-tuned transfer Learning model. *Int. J. Exp. Res. Rev.*, 31(Spl Volume), 33-41. https://doi.org/10.52756/10.52756/ijerr.2023.v31spl.004

Moynihan, K. A., Grimm, A. A., Plueger, M. M., Bernal-Mizrachi, E., Ford, E., Cras-Méneur, C., Permutt, M. A., & Imai, S. I. (2005). Increased dosage of mammalian Sir2 in pancreatic β cells enhances glucose-stimulated insulin secretion in mice. *Cell Metabolism*, *2*(2), 105–117. https://doi.org/10.1016/j.cmet.2005.07.001

Nihalani, D., & Susztak, K. (2013). Sirt1-claudin-1 crosstalk regulates renal function. *Nature Medicine*, *19*(11), 1371–1372. https://doi.org/10.1038/nm.3386

Muhammad, T., Ikram, M., Ullah, R., Rehman, S. U., & Kim, M. O. (2019). Hesperetin, a citrus flavonoid, attenuates LPS-induced neuroinflammation, apoptosis and memory impairments by modulating TLR4/NF-κB signaling. *Nutrients*, *11*(3), 1–20. https://doi.org/10.3390/nu11030648

Pawar, S., Pawade, K., Nipate, S., Balap, A., Pimple, B., Wagh, V., Kachave, R., & Gaikwad, A. (2023). Preclinical evaluation of the diabetic wound healing activity of phytoconstituents extracted from *Ficus racemosa* Linn. leaves. *Int. J. Exp. Res. Rev.*, 32, 365-377. https://doi.org/10.52756/ijerr.2023.v32.032

Peppa-Patrikiou, M., Dracopoulou, M., & Dacou-Voutetakis, C. (1998). Urinary endothelin in adolescents and young adults with insulin-dependent diabetes mellitus: Relation to urinary albumin, blood pressure, and other factors. *Metabolism: Clinical and Experimental*, *47*(11), 1408–1412. https://doi.org/10.1016/S0026-0495(98)90314-6

Price, N. L., Gomes, A. P., Ling, A. J. Y., Duarte, F. V., Martin-Montalvo, A., North, B. J., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J. S., Hubbard, B. P., Varela, A. T., Davis, J. G., Varamini, B., Hafner, A., Moaddel, R., Rolo, A. P., Coppari, R., Palmeira, C. M., … Sinclair, D. A. (2012). SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metabolism*, *15*(5), 675–690. https://doi.org/10.1016/j.cmet.2012.04.003

Purushotham, A., Schug, T. T., Xu, Q., Surapureddi, S., Guo, X., & Li, X. (2009). Hepatocyte-Specific Deletion of SIRT1 Alters Fatty Acid Metabolism and Results in Hepatic Steatosis and Inflammation.

Cell Metabolism, *9*(4), 327–338. https://doi.org/10.1016/j.cmet.2009.02.006

Qi, F., Sun, J. hao, Yan, J. qun, Li, C. mei, & Lv, X. chao. (2018). Anti-inflammatory effects of isorhamnetin on LPS-stimulated human gingival fibroblasts by activating Nrf2 signaling pathway. *Microbial Pathogenesis*, *120*, 37–41. https://doi.org/10.1016/j.micpath.2018.04.049

Roy, R., Chakraborty, A., Jana, K., Sarkar, B., Biswas, P., & Madhu, N.R. (2023). The Broader Aspects of Treating Diabetes with the Application of Nanobiotechnology. Springer Nature Singapore Pte Ltd., Advances in Diabetes Research and Management, pp. 137–162, Online ISBN-978-981-19-0027-3, https://doi.org/10.1007/978-981-19-0027-3_7

Sarkar, S., Sadhu, S., Roy, R., Tarafdar, S., Mukherjee, N., Sil, M., Goswami, A., & Madhu, N.R. (2023). Contemporary Drifts in Diabetes Management. *Int. J. App. Pharm.*, 15(2): 1-9.

Shokri Afra, H., Zangooei, M., Meshkani, R., Ghahremani, M. H., Ilbeigi, D., Khedri, A., Shahmohamadnejad, S., Khaghani, S., & Nourbakhsh, M. (2019). Hesperetin is a potent bioactivator that activates SIRT1-AMPK signaling pathway in HepG2 cells. *Journal of Physiology and Biochemistry*, *75*(2), 125–133. https://doi.org/10.1007/s13105-019-00678-4

Simms, D., Cizdziel, P., & Chomczynski, P. (1993). TRIzol: a new reagent for optimal single-step isolation of RNA. *Focus*, 99–102. http://www.invitrogen.co.jp/focus/154099.pdf%5Cnpapers2://publication/uuid/B68F1A0C-FB1A-4A64-8567-DE4CA8297D16

Song, L., Wang, K., Yin, J., Yang, Y., Li, B., Zhang, D., Wang, H., Wang, W., Zhan, W., Guo, C., Gu, Z., Wang, L., Zeng, Z., Bei, W., Rong, X., & Guo, J. (2022). Traditional Chinese Medicine Fufang-Zhenzhu-Tiaozhi capsule prevents renal injury in diabetic minipigs with coronary heart disease. *Chinese Medicine (United Kingdom)*, *17*(1), 1–14. https://doi.org/10.1186/s13020-022-00648-x

Sun, H. K., Lee, Y. M., Han, K. H., Kim, H. S., Ahn, S. H., & Han, S. Y. (2012). Phosphodiesterase inhibitor improves renal tubulointerstitial hypoxia of the diabetic rat kidney. *Korean Journal of Internal Medicine*, *27*(2), 163–170. https://doi.org/10.3904/kjim.2012.27.2.163

Sur, T., Das, A., Bashar, S., Tarafdar, S., Sarkar, B., & Madhu, N.R. (2023). Biochemical Assay for Measuring Diabetes Mellitus. Springer Nature Singapore Pte Ltd., Advances in Diabetes Research and Management, pp. 1–20. Online ISBN-978-981-19-0027-3, https://doi.org/10.1007/978-981-19-0027-3_1

Susztak, K., Raff, A. C., Schiffer, M., & Böttinger, E. P. (2006). Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes*, *55*(1), 225–233. https://doi.org/10.2337/diabetes.55.01.06.db05-0894

Thomas, M. C., Tikellis, C., Kantharidis, P., Burns, W. C., Cooper, M. E., & Forbes, J. M. (2004). The role of advanced glycation in reduced organic cation transport associated with experimental diabetes. *Journal of Pharmacology and Experimental Therapeutics*, *311*(2), 456–466. https://doi.org/10.1124/jpet.104.070672

Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461. Portico. https://doi.org/10.1002/jcc.21334

van Horssen, J., Schreibelt, G., Drexhage, J., Hazes, T., Dijkstra, C. D., van der Valk, P., & de Vries, H. E. (2008). Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. *Free Radical Biology and Medicine*, *45*(12), 1729–1737. https://doi.org/10.1016/j.freeradbiomed.2008.09.023

Wang, J., Liu, W., Luo, G., Li, Z., Zhao, C., Zhang, H., Zhu, M., Xu, Q., Wang, X., Zhao, C., Qu, Y., Yang, Z., Yao, T., Li, Y., Lin, Y., Wu, Y., & Li, Y. (2018). Synergistic effect of well-defined dual sites boosting the oxygen reduction reaction. *Energy and Environmental Science*, *11*(12), 3375–3379. https://doi.org/10.1039/c8ee02656d

Wenzel, R. R., Czyborra, P., Lüscher, T. F., & Philipp, T. (1999). Endothelin in cardiovascular control: The role of endothelin antagonists. *Current Hypertension Reports*, *1*(1), 79–87. https://doi.org/10.1007/s11906-999-0077-7

Yoshino, J., Mills, K. F., Yoon, M. J., & Imai, S. I. (2011). Nicotinamide mononucleotide, a key NAD + intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metabolism*, *14*(4), 528–536. https://doi.org/10.1016/j.cmet.2011.08.014

Yu, L., Wang, Y., Guo, Y. H., Wang, L., Yang, Z., Zhai, Z. H., & Tang, L. (2022). HIF-1α Alleviates High-Glucose-Induced Renal Tubular Cell Injury by

Promoting Parkin/PINK1-Mediated Mitophagy. *Frontiers in Medicine*, *8*, 1–8. https://doi.org/10.3389/fmed.2021.803874

Zhuo, L., Fu, B., Bai, X., Zhang, B., Wu, L., Cui, J., Cui, S., Wei, R., Chen, X., & Cai, G. (2011). NAD blocks high glucose induced mesangial hypertrophy via activation of the sirtuins-AMPK-mTOR pathway. *Cellular Physiology and Biochemistry*, *27*(6), 681–690. https://doi.org/10.1159/000330077

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