RESEARCH PAPER

Molecular Mechanism To Identify The Effects Of Seendhil Polyherbal Preparation On Irs-1/Akt/Glut4/Nrf2-Keap-1 Mediated Signaling In Sfz-Induced Type-2 Diabetic Adult Male Rats

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Abstract

Background: Type 2 diabetes mellitus (t2dm) is characterized by impaired insulin signaling, oxidative stress, and chronic inflammation.

Aim: The present study aimed to investigate the molecular effects of seenthil polyherbal formulation (sph) on the irs-1/pi3k/akt/glut4 insulin signaling pathway and the nrf2–keap1 antioxidant system in streptozotocin (stz)-induced diabetic rats.

Methods: Adult male wistar rats (n=32) were divided into four groups: control, diabetic (dm), sph-treated (500 mg/kg), and metformin-treated (50 mg/kg). Gene expression levels of insulin signaling markers (ir, irs-1, pi3k, akt, as160, glut4), antioxidant markers (nrf2, keap1), and inflammatory cytokines (il-1β, il-6, tnf-α) were analyzed using rt-pcr. Statistical significance was determined by one-way anova followed by duncan's multiple range test, with $p<0.05$ considered significant.

Results: The diabetic group showed significant downregulation of ir, irs-1, pi3k, akt, as160, and glut4 gene expression compared to control ($p<0.05$), indicating impaired insulin signaling. Sph treatment significantly upregulated these genes compared to the diabetic group ($p<0.05$), with near-normal restoration of irs-1 and glut4 expression. Similarly, nrf2 expression was significantly decreased in diabetic rats and was markedly increased following sph treatment ($p<0.05$), while keap1 expression was normalized ($p<0.05$). Pro-inflammatory cytokines il-1β, il-6, and tnf-α were significantly elevated in the diabetic group ($p<0.05$), whereas sph treatment significantly reduced their expression levels ($p<0.05$). Metformin treatment also showed significant improvements ($p<0.05$), though sph exhibited relatively stronger effects on glut4 expression.

Conclusion: Sph effectively ameliorates insulin resistance, oxidative stress, and inflammation by modulating key molecular pathways, suggesting its potential as a multi-target therapeutic agent for t2dm.

Keywords: Type 2 Diabetes Mellitus, Seenthil Polyherbal Formulation, Tinospora Cordifolia, Insulin Signaling, Irs-1/Pi3k/Akt Pathway, Oxidative Stress, Inflammation, Gene Expression.

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INTRODUCTION

Diabetes mellitus (DM) is a progressive, chronic metabolic disorder that has reached pandemic proportions, affecting approximately 537 million adults worldwide, with projections rising to 783 million by 2045, as reported by the International Diabetes Federation (2021) (1). Type 2 diabetes mellitus (T2DM), accounting for nearly 90–95% of all diabetes cases, is characterized by peripheral insulin resistance, progressive pancreatic β-cell dysfunction, and persistent hyperglycemia, ultimately leading to severe microvascular and macrovascular complications. The pathogenesis of T2DM is multifactorial, involving complex interactions between genetic predisposition, sedentary lifestyle, obesity, oxidative stress, chronic inflammation, and dysregulated intracellular signaling pathways (2,3). At the molecular level, insulin regulates glucose homeostasis through a highly coordinated signaling cascade initiated by its binding to the insulin receptor (IR), leading to receptor autophosphorylation and activation of insulin receptor substrate-1 (IRS-1). Activated IRS-1 recruits phosphatidylinositol 3-kinase (PI3K), resulting in the formation of phosphatidylinositol-3,4,5-trisphosphate (PIP_3) and activation of protein kinase B (Akt). Akt subsequently phosphorylates AS160, promoting the translocation of glucose transporter 4 (GLUT4) to the plasma membrane and facilitating glucose uptake in skeletal muscle and adipose tissue. In T2DM, this IRS-1/PI3K/Akt/GLUT4 signaling pathway is significantly impaired due to decreased IRS-1 activity and increased serine phosphorylation mediated by stress kinases such as JNK, IKKβ, and PKC, leading to insulin resistance (4,5).

Oxidative stress plays a pivotal role in the progression of T2DM. Chronic hyperglycemia enhances the production of reactive oxygen species (ROS) via mitochondrial dysfunction, advanced glycation end-product (AGE) formation, and activation of the polyol and hexosamine pathways. The nuclear factor erythroid 2–related factor 2 (NRF2)–Kelch-like ECH-associated protein 1 (KEAP1) pathway serves as the primary endogenous defense mechanism against oxidative stress. Under basal conditions, KEAP1 binds NRF2 and promotes its degradation; however, under oxidative stress, NRF2 dissociates, translocates to the nucleus, and activates antioxidant response element (ARE)-driven genes such as HO-1, NQO1, SOD, CAT, and GCLC. Dysregulation of this pathway in diabetic conditions leads to impaired antioxidant defense and increased cellular damage (6,7). Importantly, oxidative stress and insulin resistance are mechanistically interconnected. Excess ROS activates stress-sensitive kinases such as JNK and p38 MAPK, leading to inhibitory serine phosphorylation of IRS-1 and impaired insulin signaling, while defective NRF2 signaling further exacerbates oxidative damage to β-cells and peripheral tissues (8,9). This bidirectional relationship highlights the necessity of targeting both pathways simultaneously for effective therapeutic intervention. Conventional pharmacological agents for T2DM, including metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, and SGLT2 inhibitors, are widely used for glycemic control but are often associated with adverse effects such as hypoglycemia, gastrointestinal disturbances, weight gain, and cardiovascular risks (10). These limitations have prompted growing interest in alternative therapeutic strategies with multi-target mechanisms and improved safety profiles.

Traditional systems of medicine, particularly the Siddha system from South India, emphasize the use of polyherbal formulations for managing diabetes. These formulations operate on the principle of synergism, wherein multiple phytoconstituents act on diverse molecular targets simultaneously. Experimental studies have demonstrated that polyherbal formulations significantly reduce blood glucose levels, improve insulin secretion, normalize lipid profiles, and enhance antioxidant enzyme activities (11-13). Mechanistic investigations further support these effects. Haye et al. (2022) reported restoration of IRS, PI3K, Akt, and GLUT2 expression in STZ-induced diabetic rats (12). Balaji et al. (2013) demonstrated that a Siddha polyherbal formulation enhanced insulin receptor signaling and restored IRS-2, phosphorylated Akt, AS160, and GLUT2 protein expression, surpassing the effects of metformin (14). Similarly, Majeed (2018) observed significant reductions in blood glucose and improvements in insulin levels following polyherbal treatment (15). Among medicinal plants, *Tinospora cordifolia* (Seendhil) is widely recognized for its antidiabetic, antioxidant, and anti-inflammatory properties. Studies have shown that *T. cordifolia* enhances GLUT4 translocation and insulin signaling via the PI3K/Akt pathway while also activating antioxidant defenses. Sangeetha et al. (2013) reported upregulation of GLUT4 expression in L6 myotubes (16), while Sharma et al. (2019) demonstrated improved glucose uptake and β-cell protection in diabetic rats (17). Network pharmacology analysis by Khanal et al. (2019) further confirmed its multi-target therapeutic potential (18), and Patial et al. (2021) showed activation of PPARγ and protection against diabetic nephropathy (19).

The streptozotocin (STZ)-induced diabetic rat model remains a widely accepted and clinically relevant model for studying T2DM. STZ induces β-cell damage through oxidative stress and DNA alkylation, leading to hyperglycemia, insulin resistance, and metabolic dysfunction. When combined with nicotinamide or high-fat diet, this model closely mimics human T2DM, making it suitable for evaluating both therapeutic efficacy and molecular mechanisms (20). Despite extensive evidence supporting the benefits of polyherbal formulations and individual phytochemicals, there remains a significant gap in understanding their precise molecular mechanisms, particularly their dual modulation of insulin signaling and antioxidant defense pathways. No previous study has comprehensively evaluated the simultaneous effects of a Seendhil-based polyherbal formulation on both the IRS-1/PI3K/Akt/GLUT4 axis and the NRF2-KEAP1 pathway in a single experimental model. Therefore, the present study was designed to investigate the molecular mechanisms underlying the antidiabetic effects of a Seendhil polyherbal formulation in STZ-induced type 2 diabetic rats, with specific emphasis on its modulatory effects on the IRS-1/Akt/GLUT4 insulin signaling pathway and the NRF2-KEAP1 antioxidant defense system. By targeting both insulin resistance and oxidative stress, this study aims to provide a comprehensive mechanistic foundation for the development of effective, multi-targeted therapeutic strategies for T2DM management.

MATERIALS AND METHODS

Chemicals and reagents

The experiments involved chemicals and reagents are of analytical and molecular biology grade, which were obtained at BDH Laboratory Supplies (Poole, UK); Loba Chemie (Mumbai, India); TCI Chemicals (Tokyo, Japan); VWR International (Radnor, PA, USA); Fisher Scientific (Pittsburgh, PA, USA); and Carl Roth GmbH (Karlsruhe, Germany).

Poly herbal preparation

A polyherbal Siddha formulation known as Seendhil that is traditionally applied to manage diabetes mellitus and other chronic conditions of the body. This preparation is composed of a combination of ten different herbs, with (*Tinospora cordifolia*), Kadukkai thol (*Terminalia chebula*), Nellikkai vattral (*Ribes uvacrispa*), Kariveppillai elai (*Murraya koenigii*), Vilvam (*Aegle marmelos*), Manjal (*Curcuma longa*), Vendhayam (*Trigonella foenum-graecum*), Kovai elai (*Coccinia grandis*), Sirukurinjaan elai (*Gymnema sylvestre*), Maramanjal (*Berberis aristata*). To prepare, the chosen parts of the plants are acquired in the nearby markets, washed, and allowed to dry in the shade of the atmosphere in seven days. Once completely dry, a mechanical grinder is used to grind each plant material into fine powder and they are then sieved in order to obtain uniformly sized particles and then mixed in equal proportions to obtain the final polyherbal blend. The formulations are stored as representative samples of the formulation in the National Institute of Siddha, Department of Medicinal Botany, Chennai, India.

Animals

Thirty-two adult male Wistar rats were housed under controlled laboratory conditions and randomly divided into four groups (n=8), following IAEC-approved protocols. Type 2 diabetes was induced using a 30-day high-fat diet combined with a single intraperitoneal injection of streptozotocin (35 mg/kg body weight). The experimental groups included normal control, diabetic control, polyherbal-treated (300 mg/kg twice daily), and metformin-treated (50 mg/kg once daily) rats for 30 days.

Serum Insulin

Using an ultrasensitive rat insulin ELISA kit from Crystal Chem Inc. (Illinois, USA), serum insulin was measured. 0.1-64 ng/ml is the detection range. The insulin antibody's cross-reactivity with rat insulin was 100%. There was a coefficient of variation of 10.0% both within and between the assays. The results were given in mIU/ml.

Gene Expression analysis

Total RNA (2 µg) was reverse-transcribed into complementary DNA (cDNA) using a two-step RT-PCR protocol with oligo(dT) primers, dNTPs, and reverse transcriptase, followed by incubation at 37°C for 1 h. The synthesized cDNA was subjected to PCR amplification using gene-specific primers, with an initial denaturation at 95°C for 5 min, followed by cyclic denaturation (95°C, 2 min), annealing (60°C, 30 s), and extension (73°C, 30 s). The amplified products were separated on a 2% agarose gel containing ethidium bromide and electrophoresed at 80 V and 60 mA for 2 h alongside a 100 bp DNA ladder. Bands were visualized under UV illumination and captured for analysis. Gene expression levels were quantified by densitometry using Quantity One software (Bio-Rad, USA) and normalized against β-actin as the internal control.

Statistical analysis

Data analysis will employ one-way analysis of variance (ANOVA) with Duncan multiple range test to identify significant differences between the control and treatment groups. All calculations will be done using SPSS version 7.5 windows student version. The results will be reported in the form of mean +-standard error of the mean (SEM). A p-value of less than 0.05 (p < 0.05) will be considered significant.

RESULTS

Serum Insulin levels

The serum insulin levels were significantly elevated in the diabetic (DM) group (58 ± 3 µIU/mL) compared to the control group (34 ± 1.2 µIU/mL), indicating insulin resistance. Treatment with SPH (500 mg/kg) significantly reduced insulin levels (40 ± 2 µIU/mL), comparable to metformin (36 ± 2.4 µIU/mL), suggesting improved insulin sensitivity. The Control + SPH group (37 ± 1.7 µIU/mL) remained similar to control, confirming the safety of SPH (Figure 1).

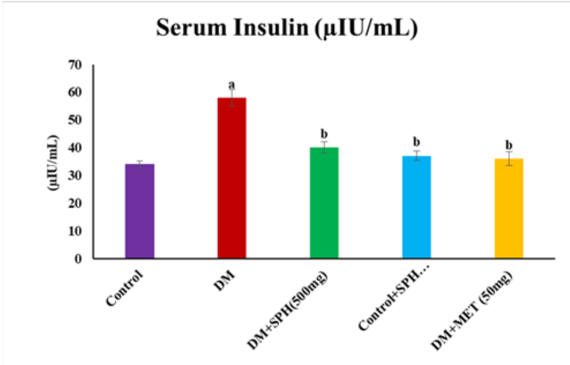

Figure 1: Effect of Seenthil polyherbal formulation (SPH) on serum insulin levels in experimental groups of type 2 diabetic rats. Values are expressed as mean ± SEM (n = 6). *a* indicates significant difference compared with control; *b* indicates significant difference compared with diabetic group (*p < 0.05*).

Effect of Seenthil on Gene Expression (RT-PCR Analysis)

Insulin Signalling Genes

It demonstrates a significant downregulation of key insulin signaling genes (IR, IRS-1, and GLUT4) in the diabetic group, indicating impaired insulin receptor function, defective downstream signaling, and reduced glucose uptake, thereby confirming the presence of insulin resistance. Treatment with Seenthil polyherbal formulation (SPH, 500 mg/kg) effectively restored the expression of these genes to near-normal levels, with complete normalization observed for IRS-1 and GLUT4, suggesting enhanced insulin sensitivity and improved glucose transport. Metformin treatment also significantly improved gene expression; however, GLUT4 recovery was slightly less pronounced compared to SPH. The Control + SPH group maintained normal expression levels, indicating the safety and possible beneficial modulatory effect of SPH under non-diabetic conditions. Overall, these findings suggest that SPH exerts potent antidiabetic effects by reactivating the insulin signaling pathway at multiple molecular levels (Figure 2).

The mRNA expression analysis revealed a significant downregulation of key downstream insulin signaling molecules, AKT, AS160 and PI3K, in the diabetic (DM) group, confirming impaired intracellular insulin signaling and disrupted glucose metabolism. Treatment with Seenthil polyherbal formulation (SPH, 500 mg/kg) markedly improved the expression of both AKT and PI3K, indicating restoration of insulin signal transduction. The Control + SPH group also exhibited enhanced expression levels, suggesting a potential beneficial modulatory effect under normal physiological conditions. Metformin treatment similarly restored AKT and PI3K expression to near-normal levels. Overall, these findings demonstrate that SPH effectively ameliorates insulin resistance by reactivating critical downstream components of the insulin signaling pathway (Figure 3).

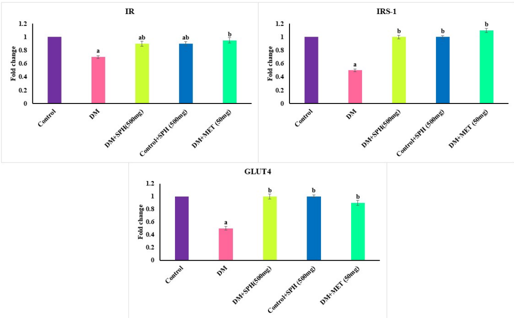

Figure 2: Relative mRNA expression of insulin receptor (IR), insulin receptor substrate-1 (IRS-1), and GLUT4 in control and experimental groups of T2DM rats. The diabetic group showed significant downregulation of all three genes compared to control ($p<0.05$), indicating impaired insulin signaling. Treatment with SPH (500 mg/kg), Control + SPH, and Metformin significantly restored IR and IRS-1 expression, while GLUT4 expression was markedly improved, with SPH showing near-complete normalization compared to slightly lower recovery with metformin.

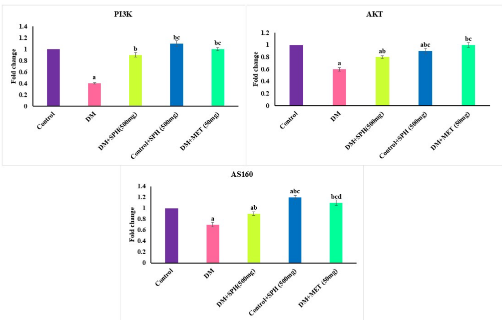

Figure 3: Relative mRNA expression of AKT, AS160 and PI3K in control and experimental groups of T2DM rats. The diabetic group showed significant downregulation of all the genes ($p<0.05$), indicating impaired insulin signaling. Treatment with SPH (500 mg/kg), Control + SPH, and Metformin significantly restored AKT, AS160 and PI3K expression toward normal levels, with Control + SPH showing slightly higher expression.

Antioxidant markers

The mRNA expression analysis of oxidative stress–related genes demonstrated a significant downregulation of Nrf2 in the diabetic (DM) group, indicating impaired antioxidant defense mechanisms under hyperglycemic conditions. Conversely, KEAP1 expression showed altered regulation in the DM group, reflecting disruption of the Nrf2–KEAP1 signaling balance. Treatment with Seenthil polyherbal formulation (SPH, 500 mg/kg) significantly upregulated Nrf2 expression and normalized KEAP1 levels, suggesting restoration of antioxidant response and redox homeostasis. The Control + SPH group exhibited near-normal or slightly enhanced expression levels, indicating a protective effect even under non-diabetic conditions. Metformin treatment also improved both Nrf2 and KEAP1 expression. Overall, these findings indicate that SPH effectively mitigates oxidative stress by modulating the Nrf2–KEAP1 antioxidant pathway (Figure 4).

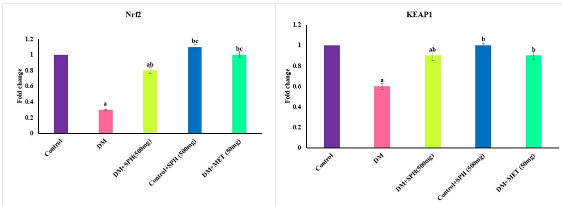

Figure 4: Relative mRNA expression of Nrf2 and KEAP1 in control and experimental groups of T2DM rats. The diabetic group showed significant downregulation of Nrf2 and altered KEAP1 expression ($p<0.05$), indicating impaired antioxidant defense. Treatment with SPH (500 mg/kg), Control + SPH, and Metformin significantly increased Nrf2 expression and restored KEAP1 levels toward normal.

Anti-inflammatory enzymes

The mRNA expression analysis revealed a significant upregulation of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in the diabetic (DM) group, indicating enhanced inflammatory responses associated with insulin resistance. Treatment with Seenthil polyherbal formulation (SPH, 500 mg/kg) significantly reduced the expression of these cytokines, restoring them toward near-normal levels and suggesting a strong anti-inflammatory effect. The Control + SPH group maintained normal expression levels, indicating no adverse effects and possible protective action. Metformin treatment also effectively attenuated cytokine expression. Overall, these results demonstrate that SPH mitigates diabetes-induced inflammation (Figure 5).

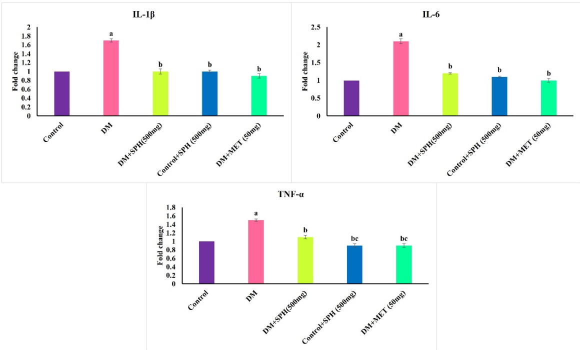

Figure 5: Relative mRNA expression of IL-1β, IL-6, and TNF-α in control and experimental groups of T2DM rats. The diabetic group showed significant upregulation of all three cytokines ($p<0.05$), while treatment with SPH (500 mg/kg), Control + SPH, and Metformin significantly reduced their expression toward normal levels.

DISCUSSION

The present study elucidates the molecular mechanisms underlying the antidiabetic effects of Seenthil polyherbal preparation (SPH) in streptozotocin-induced type 2 diabetic rats, demonstrating that SPH exerts a potent therapeutic effect through simultaneous modulation of insulin signaling, oxidative stress, and inflammatory pathways. The observed elevation in serum insulin levels in the diabetic group reflects compensatory hyperinsulinemia, a hallmark of insulin resistance wherein peripheral tissues exhibits diminished responsiveness to insulin, forcing pancreatic β-cells to increase insulin secretion (5). The significant reduction in insulin levels following SPH treatment, comparable to metformin, indicates improved insulin sensitivity and reduced β-cell burden, which is consistent with previous findings showing that polyherbal formulations enhance insulin action while reducing inflammatory mediators (21).

At the molecular level, the study revealed marked suppression of key components of the insulin signaling cascade, including IR, IRS-1, PI3K, AKT, AS160, and GLUT4, in diabetic rats, confirming disruption of insulin-mediated glucose uptake. This impairment is characteristic of T2DM, where oxidative stress and metabolic dysregulation interfere with receptor activation and downstream signaling (22). SPH treatment effectively restored the expression of these genes, with near-complete normalization of IRS-1 and GLUT4, indicating enhanced insulin receptor function and improved glucose transport. The restoration of the IRS-1/PI3K/Akt/GLUT4 axis suggests that SPH acts at multiple nodes within the signaling cascade, thereby ensuring effective propagation of insulin signals. These findings are in agreement with earlier studies demonstrating that polyherbal formulations and phytoconstituents can reactivate insulin signaling pathways and improve glucose homeostasis (12-13,23). Notably, the superior recovery of GLUT4 expression compared to metformin suggests that SPH may exert a more pronounced effect on peripheral glucose uptake, potentially due to the synergistic action of its bioactive constituents. In particular, Tinospora cordifolia, a key component of SPH, has been reported to enhance GLUT4 expression and translocation via activation of the PI3K/Akt pathway (16,17), supporting the mechanistic basis of the present findings.

Oxidative stress plays a central role in the progression of T2DM, and the significant downregulation of Nrf2 observed in the diabetic group indicates compromised antioxidant defense mechanisms. Under physiological conditions, Nrf2 regulates the expression of cytoprotective genes, but chronic hyperglycemia disrupts this pathway, leading to excessive accumulation of reactive oxygen species (ROS). SPH treatment significantly upregulated Nrf2 expression and normalized KEAP1 levels, suggesting restoration of redox homeostasis through activation of the NRF2–KEAP1 pathway. This is consistent with emerging evidence that phytochemicals exert antidiabetic effects through activation of NRF2-mediated antioxidant responses (24,25). Furthermore, genetic and pharmacological studies have demonstrated that activation of NRF2 signaling can improve insulin sensitivity and prevent the onset of diabetes (26,27). The observation that SPH enhanced Nrf2 expression even in non-diabetic conditions suggests a potential preconditioning effect, which may confer protection against oxidative stress–induced metabolic disturbances (28).

Importantly, oxidative stress and insulin resistance are closely interconnected processes. Excess ROS activates stress-sensitive kinases such as JNK and IKKβ, which induce inhibitory serine phosphorylation of IRS-1, thereby impairing insulin signaling (5). By restoring NRF2 activity and reducing oxidative stress, SPH may prevent this inhibitory modification, thereby facilitating proper insulin signal transduction. This dual modulation is further supported by studies demonstrating crosstalk between NRF2 and PI3K/Akt signaling pathways (29), highlighting the significance of targeting both pathways simultaneously. In addition to oxidative stress, chronic low-grade inflammation is a key contributor to insulin resistance in T2DM. The significant upregulation of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in the diabetic group confirms the presence of an inflammatory milieu that disrupts insulin signaling and promotes metabolic dysfunction. SPH treatment markedly reduced the expression of these cytokines, indicating a strong anti-inflammatory effect. These findings are consistent with previous reports demonstrating that polyherbal formulations attenuate inflammation by suppressing NF-κB signaling and related pathways (30,31). The reduction in TNF-α and IL-6 is particularly relevant, as these cytokines are known to impair insulin signaling through activation of JNK and SOCS-mediated pathways (32). By attenuating inflammatory signaling, SPH likely contributes to the restoration of IRS-1 function and downstream signaling events. The combined effects of SPH on oxidative stress, inflammation, and insulin signaling suggest a coordinated mechanism of action that disrupts the pathological crosstalk driving T2DM progression. By activating NRF2 and enhancing antioxidant defenses, SPH reduces ROS levels, which in turn limits the activation of stress kinases and inflammatory pathways. Simultaneously, suppression of pro-inflammatory cytokines alleviates inhibitory effects on insulin signaling, allowing restoration of the IRS-1/PI3K/Akt/GLUT4 cascade and improved glucose uptake. This integrated mechanism is supported by studies demonstrating that natural compounds can simultaneously modulate antioxidant and inflammatory pathways to improve metabolic outcomes (33).

Compared to metformin, which primarily acts through AMPK activation and suppression of hepatic gluconeogenesis, SPH exhibited comparable efficacy across most parameters and showed slightly superior effects in restoring GLUT4 expression, suggesting enhanced peripheral insulin sensitivity. The ability of SPH to target multiple interconnected pathways represents a significant therapeutic advantage over single-target drugs. Furthermore, the absence of adverse effects in the Control + SPH group indicates a favorable safety profile, supporting its potential for long-term use. In summary, the findings of this study demonstrate that Seenthil polyherbal preparation exerts its antidiabetic effects through a multi-targeted mechanism involving restoration of insulin signaling via the IRS-1/PI3K/Akt/GLUT4 pathway, activation of the NRF2–KEAP1 antioxidant defense system, and suppression of pro-inflammatory cytokines. This integrated modulation effectively interrupts the pathological interplay between oxidative stress, inflammation, and insulin resistance, providing a comprehensive therapeutic strategy for the management of type 2 diabetes mellitus.

CONCLUSION

The present study demonstrates that the Seenthil polyherbal preparation (SPH) exerts significant antidiabetic effects in STZ-induced type 2 diabetic rats through a multi-targeted molecular mechanism. SPH effectively ameliorated hyperinsulinemia and improved insulin sensitivity by restoring the IRS-1/PI3K/Akt/GLUT4 signaling cascade, thereby enhancing glucose uptake. Concurrently, SPH activated the NRF2–KEAP1 antioxidant pathway, leading to improved redox balance and protection against oxidative stress–induced cellular damage. In addition, SPH markedly attenuated chronic inflammation by downregulating pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. The integrated modulation of these interconnected pathways highlights the ability of SPH to interrupt the pathological crosstalk between oxidative stress, inflammation, and insulin resistance. Notably, the therapeutic efficacy of SPH was comparable to, and in some aspects superior to, metformin, particularly in restoring GLUT4 expression. Overall, these findings establish a strong mechanistic basis for the potential use of SPH as an effective, safe, and multi-target complementary therapeutic strategy for the management of type 2 diabetes mellitus.

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