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Original Article

Potential Molecular level Impact of Cresvin beta on Type 2 Diabetes Mellitus: A Randomized Controlled Clinical Trial

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder with an increasing prevalence rate over the past few decades. Despite the availability of medications to prevent and reduce disease severity, T2DM prevalence and incidence continue to rise annually. Understanding genetic heritage's impact on therapeutic responses is improving, with pharmacogenetics being used to better comprehend the therapeutic variability of T2DM. This study aims to compare the effects of metformin and Cresvin beta capsules containing Pterocarpus marsupium, Withania somnifera, Salacia reticulata, Gymnema Sylvestre, Curcuma longa, Vitis vinifera and Piper nigrum (Black pepper extract) on diabetic and immune-related gene expression in T2DM patients. **Methods:** Sixty patients were divided into two groups: metformin-treated (group A, n=30) and Cresvin beta -treated (group B, n=30). Anthropometric, biochemical, and hematological parameters were measured at baseline and after 3 months of treatment. Gene expression levels were analyzed using quantitative real-time polymerase chain from DNA extracted from whole blood samples. **Results:** After 3 months, metformin significantly reduced fasting blood sugar (FBS), postprandial blood sugar (PPBS), and HbA1c levels ($p<0.001$). Cresvin beta also significantly reduced FBS ($p<0.01$), PPBS ($p<0.001$), and HbA1c ($p<0.001$). Gene expression analysis showed significant changes in SIRT1, AKT, SLC2A4, IL-6, and TNF-α in both groups. **Conclusion:** The study demonstrated that Cresvin beta reduced glycemic levels and improved SIRT1, Pi3k, Akt, and SLC2A4 gene expression while decreasing IL-6 and TNF-α cytokine gene expression in T2DM patients.

Keywords: Type 2 diabetes mellitus, Metformin, Ayurveda, Cresvin beta, anti-diabetic efficacy, Sirtuin 1.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by disturbances in glucose, protein and lipid metabolism. It is caused by either a lack of insulin secretion, type 1 diabetes mellitus (T1DM) or a decreased sensitivity of the tissues to insulin, type 2 diabetes mellitus (T2DM). T2DM is one of the major chronic metabolic diseases characterized by an elevated level of glucose present in the blood and urine. Insulin is a hormone that controls the level of glucose in the human body, when it disrupts insulin homeostasis, resulting in insulin resistance leading to hyperglycemia[1]. According to the International Diabetes Federation (IDF), an estimated 537 million adults are currently living with diabetes and also predicted to increase the people with diabetes to 643 million and 783 million, respectively, in the years 2030 and 2045[2]. Generally, lifestyle modification, physical exercise and a healthy diet play a vital role in reducing the risk of diabetes. However, potent therapy and management are needed in the current scenario to reduce the risk of diabetes and its associated complications [3].

Metformin hydrochloride (Biguanides) is a colorless synthetic chemical commonly used to treat diabetes worldwide[4]. It reduces blood sugar levels through several different pathways. These include inhibiting the liver's glucose production and increasing glucose uptake and utilization by peripheral tissues (such as muscle and fat). Metformin also improves energy metabolism in these tissues by activating AMP-activated protein kinase[5]. It is also, effectively treated to lower glucose levels when combined with other drugs like sulfonylureas, insulin, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists and sodium–glucose cotransporter 2 (SGLT2) inhibitors[6]. However, patients using Metformin have some side effects like diarrhoea, nausea and/or abdominal discomfort, lactic acidosis and vitamin B12 deficiency[7].

Sirtuin 1 (*SIRT1*) is a member of the class III (NAD+-dependent) histone deacetylases (HDACs) that regulates most of the metabolic responses to calorie restriction as well as cellular functions such as genome maintenance, longevity, and metabolism[8]. *SIRT1* contributes to increasing healthy aging, including a lower risk of cardiovascular and metabolic disorders, cancer, and dementia[9].*SIRT1* enhanced ATP production and insulin secretion by directly binding with the promoter region of uncoupling protein 2 (UCP2).[10] Furthermore, *SIRT1* protects diabetic patients from diabetic vascular complications[11]. Metformin is a direct activator of *SIRT1*, and Metformin-related compounds with a biguanide functional group have the potential to enhance *SIRT1* activity. It is crucial to consider the biguanides as a novel category of *SIRT1* direct activators with a weak to moderate effect[12].

The *PI3K/AKT* signaling pathway is crucial for cell biology because it helps send growth factor signals during organismal growth and for many important cellular functions, such as maintaining glucose

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levels, breaking down fats, making proteins, and making sure cells divide and stay alive. This signaling pathway and its downstream molecules offer promising therapeutic targets for T2DM and obesity treatment[13].Both glucose and lipid metabolism are regulated by *AKT*. The glucose transporter 4 (GLUT4) is translated more efficiently when activated *AKT2* is present. This protein is typically expressed in tissues that are insulin-responsive[14].The *SLC2A4* gene, which is also known as the gene for the solute carrier family 2 member 4 gene, is responsible for encoding the GLUT4. This gene is an essential component in the treatment or prevention of insulin resistance[15]. Zhai et al. (2012) studied the effect of metformin on GLUT4. The study involved administering 500 mg of metformin to PCOS group patients three times a day for three months, starting from days 3 to 5, following menstruation, and analyzing the parameters before and after the study's three-month duration. The study found that metformin enhanced the GLUT4 gene expression in the study patients[16].

Herbal remedies have risen in popularity in recent years due to their perceived safety, efficacy, and affordability compared to synthetic therapies[17-20]. Polyherbal formulations (PHF), made up of two or more herbs, originated in Ayurvedic literature (Sharangdhar Samhita) around 1300 AD. A polyherbal composition aims to boost medicinal efficacy while limiting side effects by lowering the concentration of individual herbs in a mixture. Nowadays, Ayurveda has gained exceptional attention for preventing many diseases, and the World Health Organization (WHO) has also recognized this plant-based primary medicine[21]. Ayurvedic and traditional medicines, which rely on the ancient knowledge of the medicinal value of medicinal plants, have long been used for inflammatory and infectious diseases in many parts of Asia, including India and China.

In Ayurveda, many herbal products like *Allium sativum, trigonellafoenumgraecum, Phyllanthusamarus*, etc., have antidiabetic properties and can prevent the disease severity of T2DM. Recently, polyherbal formulations have emerged as a significant and influential treatment for various illnesses, improving patients' quality of life[22].

This study utilized Cresvin beta capsules, a polyherbal antidiabetic formulation of seven herbal ingredients, to treat T2DM. This polyherbal formulation is endowed with rich antidiabetic and antioxidant activity. The ingredients of Poly Herbal Metabolite Compound include herbal extracts of *Pterocarpus marsupium, Withania somnifera, Salacia reticulata, Gymnema Sylvestre, Curcuma longa, Vitis vinifera, and Piper nigrum* (Black Pepper extract). This study aims to evaluate the diabetic profile and molecular-level alterations in T2DM patients treated with Metformin and a Cresvin beta.

MATERIALS AND METHODS

Study participants

We included participants of both genders aged 18 to 60 with newly or recently diagnosed T2DM, fasting blood sugar (FBS) below 120 mg/dl, plasma glucose (PPBS) above 200 mg/dl, hemoglobin A1c (HbA1c) between 7 and 9.5%, and who were receiving metformin 500 mg twice daily. Based on diabetic profiles, including fasting glucose, post-prandial glucose, and HbA1c, a total of 60 T2DM patients were enrolled and divided into two groups: Metformin-treated (n=30) and Poly Herbal Metabolite Compound-treated (n=30). The study was registered with CTRI (CTRI/2022/05/042422), and all participants provided informed consent after obtaining approval from the institutional ethics committee (2914/IEC/2021). Anthropometric parameters (height, weight, hip, and waist circumference) were measured in all participants before and after treatment. Additionally, systolic and diastolic blood pressure was measured twice at baseline for each participant using a digital oscillometer blood pressure monitor (BPL 120/80 B15, Indian).

Randomization and blinding

For randomization, we employed computer random allocation software version 2.0. An independent third party maintained the secrecy of the randomization code from trial participants. Each allocation was sealed in an opaque, sequentially numbered envelope with accompanying handwritten notes. No blinding was performed."

Intervention

For this research, we divided a total of 60 participants with type 2 diabetes mellitus into two groups. One group received 500 mg of metformin twice daily after meals (Group A), whereas the other group received 500 mg of Cresvin beta (Group B) before meals. Both groups underwent laboratory testing at the time of screening and again 90 days later.

Sample collection protocol

At the project's initiation, 3 ml of Ethylene diamine tetra acetic acid (EDTA) 3 ml and 2 ml of sodium fluoride sample were collected from study participants for analysing FBS,PPBS, HbA1c and gene expression level. Similarly, after 3 months of Metformin and Cresvin beta treatment, for 3 ml of EDTA and 2 ml of sodium fluoride samples were collected for the same analyses.

DNA separation and storage

According to the manufacturer's instructions, DNA was extracted from the patient's whole blood (EDTA) samples by QIAamp DNA Blood Mini Kit (cat no. ID 51106, QIAGEN). DNA quality and quantity were measured using NanoDrop 2000 (ThermoScientific), and DNA samples were stored at-80 °C.

Gene expression by Quantitative real-time polymerase chain reaction (qRT-PCR)

Quantitative real-time PCR (qRT-PCR) was performed for specific genes using the SYBR green master mix (Cat#RR820A, TB green Premix Ex TaqTM II). PCR amplification was carried out using Rotor gen-Q (QIAGEN) with specified cycle conditions (initial cycle: 50 °C for 2 min, initial denaturation 95 °C for 15 min, denaturation 95 °C for 15 s, and annealing/extension of 60 °C for 1 min, a total of 45 cycles). The housekeeping gene *GAPDH* was used as a control gene. The list of primers used in the study is tabulated in Table 1.

Statistical analysis

Means and standard deviations of demographic characteristics were calculated. Baseline and after 3-month data were analyzed using paired t test, meanwhile between two group data were analyzed by independent t test. The statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0. A p-value of $p<0.001^{***}$, $p<0.01^{**}$ and $p<0.05^{*}$ was considered statistically significant.

RESULTS

A total of 60 subjects were enrolled and divided into two groups: the Metformin group (n=30) and the Cresvin beta group (n=30). Demographic characteristics were tabulated in Table 2. Age was not significantly different between the groups ($p<0.273$). In between the groups, at the baseline and after the treatment BMI ($p<0.218$, $p<0.225$), systolic blood pressure (SBP) ($p<0.548$, $p<305$) and diastolic blood pressure (DBP) ($p<1.00$, $p<0.50$) were non-significant. Similarly, within the groups, at baseline and after the treatment were also not observed significant in BMI ($p<0.107$, $p<0.343$), SBP ($p<0.311$, $p<1.00$) and DBP ($p<0.66$, $p<0.823$).

Table 1: List of primers.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
GAPDH	CCAACGTGTCAGTGGTGGAC	GGAGAACATACCAGGTCCCTCC
SIRT1	CGGAAACAATACCTCCACCT	CACCCCAGCTCCAGTTAGAA
Akt	GCTTCCTACTGGAGCTGTGG	TCCCTCCAAGCTATCGTCCA
SLC2A4	ACCCATGATAGGGGCACTCT	TACCCTACCCAGTACCCTGC
IL-6	CTTCGGTCCAGTTGCCTTCT	GAGATGCCGTCGAGGATGTA
TNF-α	CCCGAGTGACAAGCCTGTAG	GATGGCAGAGAGGAGGTTGAC

Table 2: Baseline characteristics of study participants.

Parameters		Metformin (n=30)	Cresvin beta (n=30)	P value
Age		45.63±6.56	47.73± 8.08	0.273
	Baseline	28.60± 4.59	27.29± 3.49	0.218
Body mass index (BMI)	**After treatment**	28.48± 4.58	27.18± 3.43	0.225
	P value	0.107	0.343	
	Baseline	127.67± 10.23	126.00± 11.14	0.548
Systolic blood pressure (SBP)	**After treatment**	123.33± 9.78	126.00± 10.20	0.305
	P value	0.311	1.00	
	Baseline	81.67± 6.87	81.67± 8.60	1.00
Diastolic blood pressure (DBP)	**After treatment**	81.00± 6.51	82.00± 4.76	0.50
	P value	0.66	0.823	

Table 3: Correlation of clinical parameters in the study participant.

Parameters		Metformin (n=30)	Cresvin beta (n=30)	P value
	Baseline	183.34±29.21	171.86±34.09	0.166
Fasting blood sugar (FBS)	After treatment	151.44±27.09	121.17±18.68	0.001***
	P value	0.001***	0.001***	
	Baseline	236.51±44.28	239.03±48.62	0.834
Post prandial blood sugar (PPBS)	After treatment	198.96±26.99	190.37±23.33	0.192
	P value	0.001***	0.001***	
	Baseline	9.09±0.68	9.15±0.67	0.731
Glycated haemoglobin (HbA1c)	After treatment	8.37±0.68	7.73±0.66	0.001***
	P value	0.001***	0.001***	

***p<0.001 is statistically significant

Table 4: Correlation between gene expressions of the study groups.

Parameters		Metformin (n=30)	Cresvin beta (n=30)	P value
	Baseline	32.21±0.35	32.47±0.31	0.001***
SIRT1	After treatment	30.32±0.34	30.35±0.33	0.730
	P value	0.001***	0.001***	
	Baseline	32.74±0.45	32.60±0.34	0.179
AKT	After treatment	31.80±0.54	31.85±0.47	0.641
	P value	0.001***	0.001***	
	Baseline	32.41± 0.23	32.56± 0.23	0.01**
SLC2A4	After treatment	30.22± 0.34	31.22± 0.37	0.001***
	P value	0.001***	0.001***	
	Baseline	30.64± 0.34	31.43± 0.25	0.001***
IL-6	After treatment	32.85± 0.23	32.41± 0.26	0.001***
	P value	0.001***	0.001***	
	Baseline	30.98± 0.47	31.50± 0.63	0.001***
TNF- alpha	After treatment	32.64± 0.35	32.20± 0.57	0.003**
	P value	0.001***	0.001***	

Table 3 presents the clinical parameters association between Metformin group and Cresvin beta group. At the baseline, FBS (183.34±29.21&171.86±34.09; p<0.166), PPBS (236.51±44.28&239.03±48.62; p<0.834), and HbA1c (9.09±0.68&9.15±0.67; p<0.731) were not significantly different between the two groups. After treatment, FBS (151.44±27.09 &121.17±18.68; p<0.001) and HbA1c (8.37±0.68 &7.73±0.66; p<0.001 were significantly different between the Metformin group and Cresvin beta group, while PPBS levels were not (198.96±26.99 &190.37±23.33; p<0.192). within the Metformin group and Cresvin beta group, FBS (p<0.001, p<0.001), PPBS (p<0.001, p<0.001) and HbA1c (p<0.001, p<0.001) were significantly different between baseline and post-treatment measurements.

The gene expression of the study participants at baseline and after a 3-month follow-up is shown in Table 4. In a comparison between the two groups, baseline *SIRT1* gene CT values (32.21±0.35 vs. 32.47±0.31; p<0.001) were slightly higher in both groups. After the 3-month follow-up, *SIRT1* gene CT values (30.32±0.34 vs. 30.35±0.33; p<0.730) were lower compared to baseline. A higher CT value indicates lower gene expression. Additionally, within each group, SIRT1 gene expression was significantly different between baseline and the 3-month follow-up (p<0.001 for both groups). Furthermore, the gene expression of the study samples is presented as fold change values graphically in Figure 1.

The *AKT* gene expression of the study participants was not significantly different between the two groups at baseline (32.74±0.45 vs. 32.60±0.34; p<0.179) or after the 3-month treatment (31.80±0.54 vs. 31.85±0.47; p<0.641). However, within each study group, *AKT* gene expression was significantly different between baseline and the 3-month follow-up (p<0.001 for both groups). Furthermore, the gene expression of the study samples is presented as fold change values graphically in Figure 1.

The SLC2A4 gene expression was significantly different between the Metformin and Cresvin beta groups at baseline (32.41±0.23 vs. 32.56±0.23; p<0.01) and after treatment (30.22±0.34 vs. 31.22±0.34; p<0.001). Within both groups, SLC2A4 gene expression was also significantly different between baseline and post-treatment (p<0.001 for both groups). Gene expression fold changes are graphically represented in Figure 1.

IL-6 gene expression was not significantly different between the Metformin and Cresvin beta groups at baseline (30.64±0.34 vs. 31.43±0.25; p<0.001) or after treatment (32.85±0.23 vs. 32.41±0.26; p<0.001). However, within both groups, *IL-6* gene expression was significantly different between baseline and post-treatment (p<0.001 for both groups). Gene expression fold changes are graphically represented in Figure 1.

TNF-α gene expression was not significantly different between the Metformin and Cresvin beta groups at baseline (30.98±0.47 vs. 31.50±0.63; p<0.001) but was significantly different between the groups after treatment (32.64±0.35 vs. 32.20±0.57; p<0.01). Within both groups, *TNF-α* gene expression was significantly different between baseline and post-treatment (p<0.001 for both groups). Gene expression fold changes are graphically represented in Figure 1.

We evaluated the fold change value in pharmacogenetics analysis among the individuals in our study. The Cresvin beta -treated group exhibited a progressive increase in gene expression compared to the metformin-treated group. Cresvin beta progressively increased the expression of the Sirt1 gene compared to metformin in both groups. Similarly, both groups demonstrated growth in the AKT and SLC2A4 genes. On the other hand, when compared to the baseline, the Cresvin beta group showed enhanced gene expression in the study individuals compared to the metformin group. Additionally, both groups experienced a reduction in immunogenic genes such as IL-6 and TNF alpha; however, the Cresvin beta -treated group showed a more significant reduction in these genes compared to the metformin-treated group. Comparison of gene expression in the study group was graphically expressed in Figure 2.

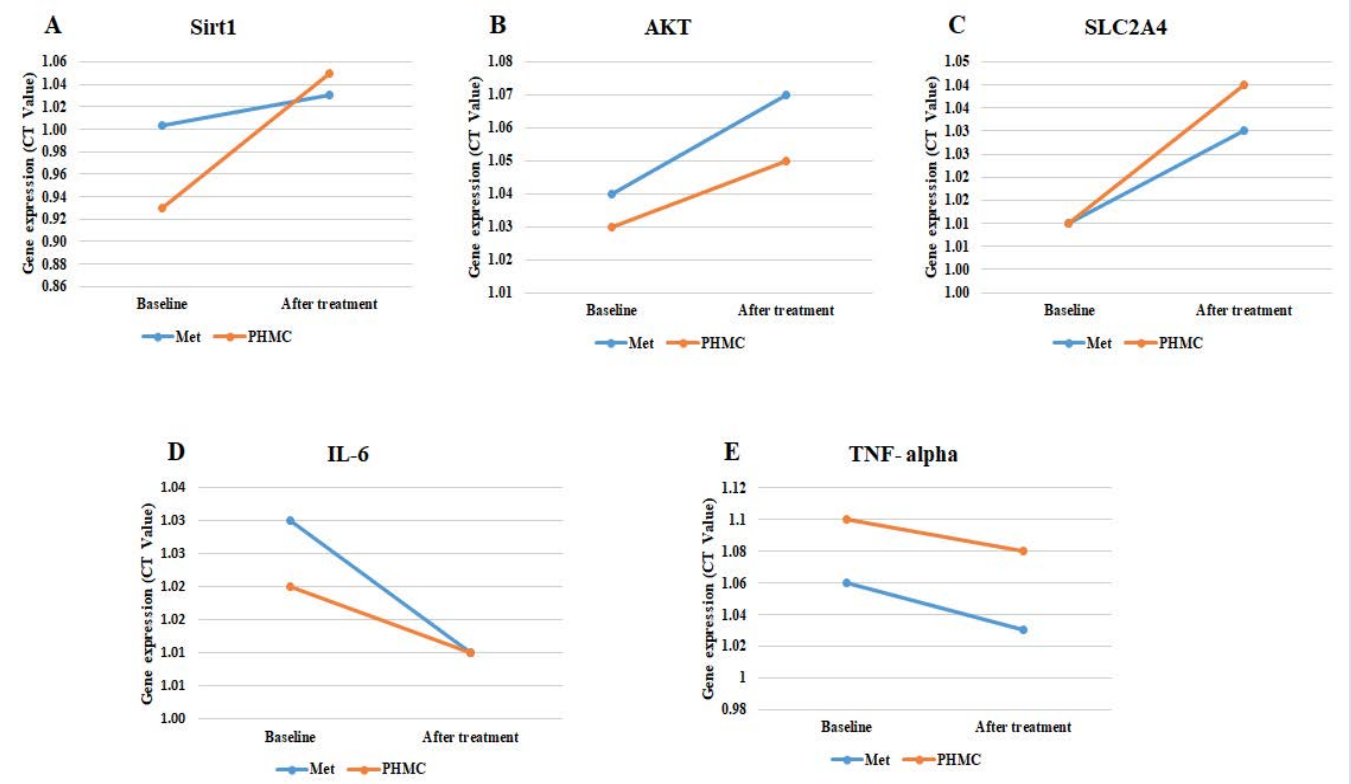

Figure 1: Relative gene expression between the study groups (a) Sirtuin 1; (b) AKT; (c) SLC2A4; (d) TNF-α and (e) IL-6.

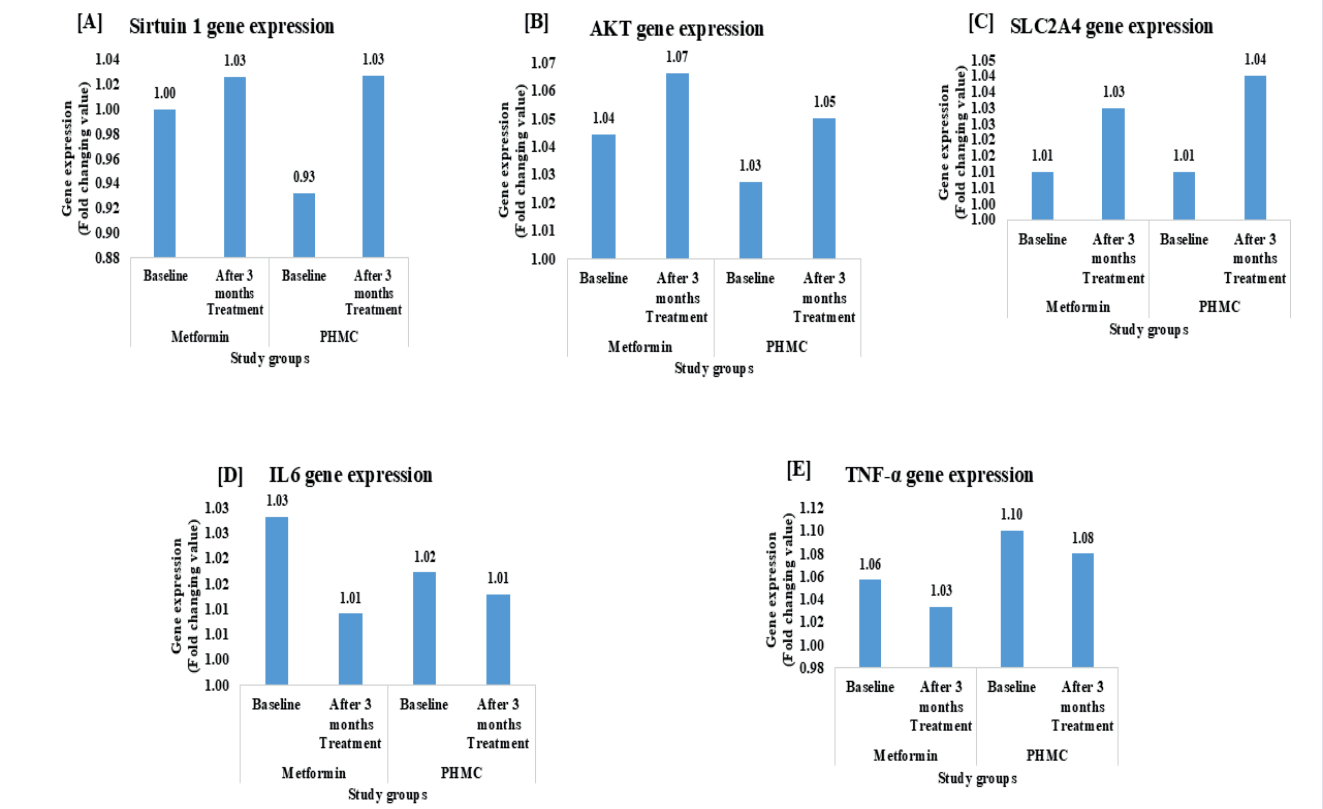

Figure 2: Comparison of gene expression in the study groups (a) Sirtuin 1; (b) AKT; (c) SLC2A4; (d) TNF-α and (e) IL-6.

DISCUSSION

The prevalence of diabetes mellitus has increased worldwide. The IDF (International Diabetes Federation) estimates that by 2045, 12.2% (783.2 million) of people aged 20 to 79 will have diabetes, an increase from 10.5% (536.6 million) in 2021 [23]. Currently, metformin is the primary oral antidiabetic drug prescribed for T2DM, either alone or in combination with thiazolidinediones, sulfonylureas, or other hypoglycemic agents[4]. However, diabetes remains a significant health concern. In the United States, it was the eighth leading cause of death in 2021, with 103,294 deaths attributed to diabetes[24]. Moreover, the mortality rate due to diabetes increased from 22.30 deaths per 100,000 in 1990 to 27.35 deaths per 100,000 in 2019[25]. Given the increasing burden of diabetes, there is a need for effective treatment options beyond Metformin.

Medicinal plants have numerous chemical compounds or phytochemicals that have different pharmacological actions. The usefulness of phytochemicals and their bioactive constituents as natural modifying agents in the remedy of numerous ailments. Medicinal plants include major phytochemical compounds with health advantages, including alkaloids, phenolics, flavonoids, terpenoids, and miscellaneous compounds. These compounds are important in ameliorating diabetes progression[26-28]. This study established the molecular level effects of Ayurvedic polyherbal components on T2DM.

Our findings demonstrate that both Metformin and Cresvin beta significantly reduced fasting and 2-hour postprandial blood glucose, as well as HbA1c levels. These findings align with those of Piera-Mardemootoo et al., 2021, who reported that metformin significantly decreased fasting, postprandial blood glucose, and HbA1c levels[4]. Furthermore, Cresvin beta components, including *Pterocarpus marsupium*[29], *Withania somnifera*[30], *Salacia reticulata*[31], *Gymnema sylvestre*[32], *Curcuma longa*[33], *Vitis vinifera*[34], and *piper nigrum (black pepper extract)*[35] also contributed to reducing blood glucose and HbA1c levels in the study participants.

Sirt1 plays a crucial role in management of T2DM[36]. Cuyàs et al. (2018) investigated the effect of metformin on *SIRT1* gene expression and found that metformin biochemically enhanced *SIRT1* efficiency[37]. Similarly, our study observed increased *SIRT1* levels in both Metformin- and Cresvin beta -treated patients. Yang et al. (2013) reported that *Curcuma longa* significantly enhanced elevated mitochondrial superoxide dismutase activity through *SIRT1* activation[38]. Moreover, *Vitis vinifera* contains the SIRT1 gene sequence and is highly prevalent in grapevines[39]. Additionally, *piperine*, a component of *piper nigrum*, has been shown to improve cognitive function in Alzheimer's patients by enhancing *SIRT1* activation[40]. Our study suggests that the Cresvin beta compound, containing *Curcuma longa, Vitis vinifera, and piper nigrum*, significantly improved *SIRT1* gene expression in Cresvin beta -treated patients, comparable to the effects of metformin.

The *SLC2A4* gene encodes the GLUT4 protein, which is crucial for facilitating glucose uptake by muscles. GLUT4 expression is essential for glucose removal from the bloodstream and blood sugar regulation. Insulin resistance is characterized by reduced GLUT4 expression[41]. The *PI3K/Akt* pathway plays a crucial role in glucose regulation by controlling glucose transport mechanisms in the blood, particularly through GLUT4[42]. Our study revealed lower *SLC2A4* gene expression in diabetes patients before treatment with both Metformin and Cresvin beta. However, *SLC2A4* gene expression significantly increased in diabetes patients after treatment with both Metformin and Cresvin beta. *Pterocarpus marsupium*, a component of PHMC, activates the glucose transporter through the *PI3K/Akt* pathway[43]. Singh et al. (2018) found that *Salacia oblonga* has a strong binding affinity for peroxisome proliferator-activated receptor gamma (PPARγ) and GLUT4. In

animal models, *Salacia oblonga* significantly increased GLUT4 levels[44]. Similarly, Kumar et al. (2016) demonstrated increased *PPARγ* and GLUT4 gene expression with methanolic leaf extract of *Gymnema sylvestre* (MLGS)[45]. Our study also showed a significant increase in *GLUT4* gene expression in patients after treatment with Metformin and Cresvin beta.

Numerous research studies have demonstrated that immune response genes, including *IL-6* and *TNF-α*, are elevated in diabetic patients[46, 47]. Our study similarly found elevated levels of *IL-6* and *TNF-α* gene expression in diabetic patients. However, both IL-6 and *TNF-α* gene expression levels were significantly reduced after treatment in both groups. Hyun et al 2013 studied the effect of metformin on inflammatory markers and especially metformin reduced the *IL-6* and *TNF-α*gene expression in mice[48]. Our study also observed reduced *IL-6* and *TNF-α* gene expression in the Metformin-treated group. Interestingly, the Cresvin beta -treated group also exhibited decreased *IL-6* and TNF-α gene expression. *S. reticulata* extract, a component of Cresvin beta, has been shown to improve immune function and alter the intestinal microbiota in humans[49]. Jangam et al. (2023) reported that a hydroalcoholic extract of Gymnema sylvestre reduced cytokines by modulating the *NF-κB/MAPK* pathway in rats[50]. Additionally, Curcuma longa possesses anti-inflammatory and antioxidant properties, effectively reducing inflammatory markers like *IL-6* and *TNF-α* in animal module[51].

CONCLUSION

The present study demonstrated the beneficial effect of this Ayurvedic Cresvin beta capsules formulation containing *Pterocarpusmarsupium, Withaniasomnifera, Salacia reticulate, GymnemaSylvestre, Curcuma longa, Vitis vinifera and piper nigrum* (Black Pepper extract) in reducing blood glucose and HbA1c levels in the T2DM participants. Moreover, this herbal combination enhanced the expression of *SIRT1, Akt* and *SLC2A4* genes while reducing the cytokines gene expression such as *IL-6* and *TNF-α* in T2DM patients.

AUTHOR CONTRIBUTIONS

Conceptualization: V.P., V.S.S., and S.R.C; Methodology: N.K.N and B.M; Software: P.S and M.N.; Validation: N.K.N., P.S., and M.N; Formal Analysis: N.K.N and B.M; Investigation: N.K.N and B.M; Resources: S.R.C and B.M; Data Curation: N.K.N., M.N and P.S; Writing – Original Draft Preparation: N.K.N., M.N., and P.S; Writing – Review & Editing: All authors; Visualization: N.K.N., M.N and P.S; Supervision: V.P and S.R.C; Project Administration: N.K.N., V.S.S., M.N., B.M and P.S.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of SRM Medical College Hospital and Research Centre (Approval Number: 2914/IEC/2021). Additionally, the trial was registered with the Clinical Trials Registry - India (CTRI/2022/05/042422).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study. Prior to their participation, all participants were provided with comprehensive information about the study's purpose, procedures, potential risks, and benefits. This information was delivered both verbally and in writing.

DATA AVAILABILITY STATEMENT

No additional data beyond what is presented in this manuscript are available. All relevant data are included within the article.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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