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Original Article

Therapeutic Effects of a Polyherbal Formulation on Metabolic Dysregulation in Type 2 Diabetic Rats by LCMS

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ABSTRACT

Aim and Objectives: This study examined the effects of a polyherbal formulation (composite of *Camellia sinensis, Cinnamomum verum, Syzygium aromaticum, Curcuma longa*, and *Elettaria cardamomum*) on lipid and glucose metabolites in an animal model of Type 2 Diabetes Mellitus (T2DM). The aim was to employ a comprehensive metabolomic approach to explore the importance of metabolic changes in Type 2 Diabetes Mellitus and to evaluate the potential modulatory effects of these herbs on the metabolic pathways. **Materials and Methods:** Individual ethanolic extraction of these herbs were combined in a predefined ratio (2:2:1:1:1) to formulate a polyherbal compositionT2DM) was experimentally induced in male Wistar rats using STZ (50 mg/kg, i.p.), administered three days after and seven days following pretreatment with nicotinamide (120 mg/kg, p.o., once daily). After the confirmation of hyperglycemia (>200 mg/dL), treatment with the polyherbal formulation and standard drug (Glibenclamide, 0.25 mg/kg) was administered orally for 21 days. Blood samples were taken at the end of the treatment, and plasma was separated for testing. LCMS analysis was performed to quantify changes in glucose and lipid metabolites. **Results:** Significant improvements in glucose and lipid metabolites were observed. In glucose metabolites; G6P, F6P, pyruvate, and lactate showed normalization trends suggesting increased mitochondrial activity and glycolytic flux. There was a significant regulation of the levels of lipid metabolites, such as oleic acid, palmitic acid, arachidonic acid, linoleic acid, phosphatidylcholine, and acylcarnitines (C16 and C18). Enhancement in cellular respiration and metabolic balance was further supported by improvements in TCA cycle intermediates, such as malate, citrate, and α-ketoglutarate. **Conclusion:** These findings show that polyherbal formulations can be a promising supplemental strategy in the treatment of type 2 diabetes and offer scientific backing for the traditional usage of herbs.

Keywords: Diabetes, Glucose and Lipid Metabolites, LCMS, Polyherbal formulation.

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a progressive metabolic disorder characterized by persistent hyperglycemia resulting from inadequate insulin secretion by pancreatic β-cells and impaired responsiveness of insulin-sensitive tissues.[1] Globally, approximately 10% of individuals aged 20-79 is affected by T2DM, making it a major public health concern. According to World Health Organization (WHO) projections, the prevalence of T2DM is expected to reach 784 million by 2045.[2] Beyond its rising incidence, T2DM is associated with chronic complications such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases, which significantly contribute to increased healthcare costs.[3]

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A key factor in the pathophysiology of type 2 diabetes is the imbalance of lipid and glucose metabolism. Elevated levels of important glucose metabolites, including lactate, pyruvate, fructose-6-phosphate, and glucose-6-phosphate, are indicative of increased glycolysis and changed mitochondrial activity in diabetic circumstances.[4] Similarly, alpha-ketoglutarate, malate, and citrate intermediates of the tricarboxylic acid cycle frequently exhibit dysregulation, which is indicative of compromised oxidative metabolism and energy imbalance.[5] Conversely, diabetics often have higher levels of free fatty acids, such as oleic acid, arachidonic acid, linoleic acid, and palmitic acid, which are linked to systemic inflammation, insulin resistance, and lipotoxicity.[6] Changes in complex lipid levels, including phosphatidylcholine and acylcarnitines (C16, C18), also indicate a breakdown in mitochondrial β-oxidation and a disturbance in lipid homeostasis.[7]

Impact of Polyherbal Formulations in Type 2 Diabetes Mellitus (T2DM)

Combinations of several medicinal plants in polyherbal preparations are becoming more widely acknowledged as

potential supplements or substitutes for traditional treatments in the treatment of various ailments, including type 2 diabetes. These plants have a variety of beneficial impacts on insulin sensitivity, inflammation, oxidative stress, glucose and lipid metabolism, and more by utilizing the synergistic effects of many bioactive substances. T2DM is characterized by chronic hyperglycemia resulting from insulin resistance and/or β-cell dysfunction. Multiple molecular pathways are simultaneously targeted by polyherbal methods to treat these complicated diseases. For instance, plant components like green tea, *Camellia sinensis* (family Theaceae), are widely cultivated in various countries. It is well recognized for its diverse pharmacological effects, including antioxidant, anticancer, hypoglycemic, antiviral, antibacterial, and neuroprotective properties.[8] *Syzygium aromaticum (clove,* Myrtaceae family) is widely used in traditional medicine and food preservation due to its potent antioxidant, antibacterial, anti-inflammatory, and antidiabetic effects.[9] *Cinnamomum verum* (family Lauraceae) is extensively studied for its anti-hyperglycaemic activity and is used in managing metabolic disorders, including diabetes.[10] *Curcuma longa*, in the Zingiberaceae family, has been a staple in Ayurvedic and Chinese medicine for centuries. Its active component, curcumin, demonstrates notable anti-inflammatory, anticancer, and antidiabetic effects.[11] Similarly, cardamom (*Elettaria cardamomum,* Zingiberaceae family) is used in the treatment of various ailments such as digestive issues, gum infections, asthma, and cardiovascular conditions. It exhibits antioxidant, anti-inflammatory, anticancer, and antidiabetic properties, making it a valuable component in polyherbal therapies.[12]

A comprehensive and culturally integrated approach is offers by polyherbal therapy, which is particularly beneficial in areas where traditional medicine is extensively used. Therefore, a polyherbal formulation was experimentally tested in a rat model of type 2 diabetes induced by STZ-NA to determine its impact on lipid and glucose metabolic markers by LCMS.

MATERIALS AND METHODS

Collection and authentication of Plant materials

The herbs materials were procured from Bangalore, Karnataka, India. And authentication and identification of the herb's material was done by the Central Ayurveda Research Institute, Bangalore. Reg. no. Authentication/SMPU/CARI/BAG/2025-26/350.

Preparation and Extraction of Herbs

The herbs were shade-dried at room temperature to preserve their phytoconstituents. The dried materials were coarsely powdered using a mixer grinder. Soxhlet extraction was performed using 99% ethanol for 3 continuous cycles of 2 hr each. The obtained extracts were then concentrated and dried at room temperature for subsequent formulation.

Formulation of Polyherbal Mixture

The ethanolic extracts of the five herbs were blended in a specific ratio of 2:2:1:1:1 for *Camellia sinensis, Cinnamomum verum, Syzygium aromaticum, Curcuma longa*, and *Elettaria cardamomum*, respectively, based on their traditional usage and reported antidiabetic potential.

Acute Toxicity and Dose Selection

Acute oral toxicity testing of the polyherbal formulation was conducted as per the OECD Guideline No. 425 (Up-and-Down Procedure). The maximum tolerable dose was determined to be 5000 mg/kg, b.w., with no mortality or signs of toxicity. Therefore, $1/10^{th}$ (500 mg/kg, b.w.) and $1/20^{th}$ (250 mg/kg, b.w.) of the dose were selected in the current study.

Induction of T2DM in experimental animals

Diabetes was induced in rats according to the method described by *Aboonabi et al.* (2014), with slight modifications. Nicotinamide (120 mg/kg, p.o.), dissolved in normal saline, was administered once daily for 7 days prior to and 3 days following the injection of Streptozotocin (50 mg/kg, i.p.), which was freshly prepared in 0.05 M citrate buffer (pH 4.5).[13]

The confirmation of diabetes involved measuring FBS levels both prior to and 72 hr after the STZ injection, with significantly elevated glucose levels indicating the onset of Type 2 Diabetes Mellitus (T2DM).

Experimental Design and Grouping

In this study, Wistar male rats (150 to 200 g, 8 to 10 weeks old) were used. The animals were kept for adaptation and adjustment prior to the experiment. All experimental procedures were carried out as per the guidelines of the CPCSEA, Government of India.

Group 1 - Normal Control

Received normal saline (2 mL/kg, p.o.) throughout the study period.

Group 2 - Diabetic Control

Induced with Nicotinamide (120 mg/kg, p.o. Nicoglow, Tricos dermatologics) administered 7 days before and for 3 days after Streptozotocin (STZ, 50 mg/kg, i.p.) injection.

Group 3 - Standard Drug Group

After T2DM confirmation, rats received Glibenclamide (0.25 mg/kg, p.o.) once daily for 21 days.

Group 4 - Polyherbal Formulation Low Dose

After T2DM confirmation, rats received Polyherbal formulation (250 mg/kg, p.o.) once daily for 21 days.

Group 5 - Polyherbal Formulation High Dose

After T2DM confirmation, rats received Polyherbal formulation (500 mg/kg, p.o.) once daily for 21 days.

Estimation of metabolomic profiling of polar and lipid metabolites by LCMS

Plasma samples were collected in EDTA-coated tubes and centrifuged at 3k × g for 15 min at 4°C to separate the supernatant, stored at -20°C until analysis.

For sample preparation

In the case of polar metabolites such as glucose and their metabolites, 50 µL of plasma is mixed with 400 µL of 2% HCl in methanol. The mixture is vortexed thoroughly for 5 min and centrifugation at 10k × g for 15 min at 4°C. The clear supernatant is then transferred to a clean tube and dried using nitrogen gas or vacuum centrifugation. Prior to LC-MS injection, the dried residue is reconstituted in 150 µL of 50% methanol in water load to LCMS. For lipidomic analysis targeting lipid metabolites, 50 µL of plasma is mixed with 400 µL of 2% HCl in methanol. The mixture is vortexed thoroughly for 5 min and centrifugation at 10k × g for 15 min at 4°C. The lower organic phase, which contains the lipids, is carefully separated, dried under nitrogen gas, and reconstituted in a 1:1 mixture of isopropanol and methanol containing 10 mM ammonium formate. Used system LCMS - Sciex 4000 Software analyst 1.7 version.[14]

Statistical Analysis

In this study, Pearson correlation (r) was used to assess relationships between metabolite pairs, supported by correlation matrices and network diagrams, with multiple testing correction applied to control for false positives. PCA was employed to visualize separation between diabetic and control groups. Fold change analysis quantified the magnitude and direction of metabolite alterations, while network metrics optionally identified hub metabolites that may act as regulatory nodes in diabetes-related metabolic pathways. The analysis was conducted using a combination of tools: MetaboAnalyst for PCA, correlation matrices, and fold change analysis, GraphPad Prism, Cytoscape for network diagram construction, and R/Python for correlation calculations.

RESULTS

After confirmation of hyperglycemia (>200 mg/dL), treatment was initiated in the respective groups. Administration of the polyherbal extract at a dose of 500 mg/kg b.w. produced a statistically significant reduction ($p<0.01$) compared with the control group. At the end of the experiment, blood samples were collected, and plasma was separated for metabolite analysis.

Lipid Metabolites with T2DM in Experimental Rats

In LC-MS analysis, Q1 refers to the mass/charge (m/z) of the precursor ion (intact molecule), Q3 refers to the product ion (fragment), both measured in Daltons (Da), and intensity values (cps) reflect the abundance of each metabolite.

In this experimental, lipid metabolite profiling revealed distinct metabolic patterns across groups. The Normal Control (NC) group served as a baseline with balanced levels of fatty acids and acylcarnitines, indicating healthy lipid metabolism. In contrast, the diabetic control group showed a marked elevation in acylcarnitines C16 (palmitoylcarnitine) and C18 (stearoylcarnitine), along with reduced fatty acids, suggesting impaired mitochondrial β-oxidation and dysfunctional fatty acid metabolism. Treatment with the Standard Drug (STD) partially normalized these abnormalities, particularly reducing acylcarnitine levels. The Polyherbal formulation (250 mg/kg, p.o.) treatment group exhibited a poor response, as fatty acids remained low and acylcarnitines stayed elevated, reflecting limited therapeutic benefit. The Polyherbal formulation (500 mg/kg, p.o.) group showed the most significant metabolic improvement, with fatty acid levels rebounding and acylcarnitine concentrations decreasing substantially, indicating restored mitochondrial function and improved lipid metabolism (refer to Table 1). Correlation between the metabolites and T2DM experimental rats were mentioned in Table 2.

Log2 fold change analysis of metabolite intensities compared to the NC reveals key metabolic shifts in the T2DM experimental groups. Red shades indicate upregulation, while blue shades reflect downregulation, with darker tones signifying larger changes. Acyl carnitines C16 and C18 show marked upregulation in the Diabetic Control (DC), consistent with mitochondrial dysfunction, but levels normalize significantly in the Polyherbal formulation (500 mg/kg, p.o.) treatment group, indicating metabolic recovery. Palmitic, oleic, and linoleic acid are notably downregulated in DC and Polyherbal formulation (250 mg/kg, p.o.) groups, with partial restoration in 500 mg/kg formulation, suggesting improved lipid metabolism. In contrast, Phosphatidylcholine remains relatively stable across conditions except the 250 mg/kg formulation indicating it may be less affected by the diabetic condition (refer to Figure 1).

Glucose Metabolites with T2DM in Experimental Rats

In our study, the metabolic profile reveals distinct differences across groups. The Normal Control group shows normal glycolysis, low lactate levels, and a balanced TCA cycle, reflecting healthy energy metabolism. In contrast, the DC group displays a complete block in early glycolysis, evidenced by the absence of G6P and F6P. This is accompanied by high lactate levels and a partly stalled TCA cycle, indicating a shift toward anaerobic metabolism and poor mitochondrial function. The Standard Drug group exhibits partial glycolytic activity with elevated F6P and a further increase in

lactate, suggesting the drug is only partially effective in restoring normal metabolism. The Polyherbal formulation (250 mg/kg, p.o.) treatment group shows partial restoration of glycolysis but extremely high lactate and pyruvate levels, indicating excessive glycolytic flux with weak TCA engagement. This overflow of pyruvate into lactate highlights inefficient oxidative metabolism. In contrast, the Polyherbal formulation (500 mg/kg, p.o.) group demonstrates significant metabolic recovery. G6P and F6P levels are restored, and TCA cycle intermediates like malate and citrate are elevated. Pyruvate and lactate are both increased but in a more balanced ratio, reflecting a shift back toward aerobic metabolism and effective energy production (refer to Tables 3 and 4).

The heatmap of metabolite intensities (with blue indicating low and red indicating high levels) clearly illustrates distinct metabolic patterns across the groups. The Diabetic Control group shows marked suppression of early glycolysis intermediates and citrate, highlighting a breakdown in both glycolysis and the TCA cycle, indicative of impaired energy metabolism. In contrast, the Polyherbal formulation (500 mg/kg, p.o.) treatment group exhibits a rebalancing of metabolite levels, with a pattern that closely resembles the Normal Control group, suggesting restoration of both glycolytic and mitochondrial function (refer to Figure 2). The Standard Drug group presents an intermediate profile, with partial recovery of key metabolites, indicating a moderate therapeutic effect and partial metabolic rescue.

Similarly, Principal Component Analysis (PCA) reveals clear metabolic distinctions between the experimental groups. The Polyherbal formulation (500 mg/kg, p.o.) group clusters closely with the Normal Control group, indicating that 500 mg/kg formulation treatment effectively restores the metabolic profile toward a healthy state. In contrast, the Diabetic Control, Standard Drug, and Polyherbal formulation (250 mg/kg, p.o.) groups are metabolically distinct and distant from NC on the PCA plot (refer to Figure 3). This separation reflects disrupted glycolysis and impaired TCA cycle activity, confirming that these groups retain significant metabolic dysfunction.

Correlation Glucose and TCA metabolites with lipid metabolites from the LC-MS data

The LC-MS data reveals strong correlations between glucose metabolism, TCA cycle intermediates, and lipid markers, which differ significantly across diabetic and treated groups. In the Diabetic Control group, there is a clear suppression of glycolysis, with undetectable levels of G6P and F6P, confirming early-stage blockage in glucose metabolism. Despite elevated pyruvate and lactate, the high lactate-to-pyruvate ratio indicates a shift toward anaerobic metabolism. TCA cycle disruption is evidenced by decreased citrate and elevated α-Ketoglutarate (AKG), suggesting incomplete cycle turnover. Lipid metabolism is also altered: Free Fatty Acids (FFAs) are reduced, while acylcarnitines (C16, C18) are elevated, implying an overload in fatty acid β-oxidation and resulting in mitochondrial stress due to incomplete oxidation. Elevated Phosphatidylcholine (PC) may reflect increased membrane remodeling or inflammation, common in diabetic pathology. In contrast, the Polyherbal formulation (500 mg/kg, p.o.) treatment group shows significant metabolic recovery. Restoration of G6P and F6P levels indicates normalization of glycolysis. Although lactate and pyruvate are still elevated, their balanced ratio and increased citrate and malate suggest recovery of TCA cycle activity and enhanced oxidative metabolism. Notably, acylcarnitine levels are reduced, reflecting improved mitochondrial fatty acid oxidation efficiency. FFAs and PC return to near-normal levels, indicating restored lipid homeostasis and reduced cellular stress (refer to Table 5). These findings collectively support that Polyherbal formulation (500 mg/kg, p.o.) treatment not only reestablishes glucose and TCA cycle activity but also alleviates lipid metabolic disturbances and mitochondrial burden observed in diabetes.

The Pearson correlation coefficient (r) provides insight into the strength and direction of associations between metabolites: r = ±0.7 to 1.0 indicates a strong correlation, r = ±0.3 to 0.7 suggests a moderate correlation, r = 0 to ±0.3 reflects a weak or negligible correlation. Red, close to +1: suggests shared biosynthetic or regulatory pathways, possibly coordinated lipid metabolism. Blue, close to -1: Suggests that high glucose metabolism may be inversely related to fatty acid oxidation or lipid turnover. Malate, Citrate, and TCA intermediates have consistently strong positive correlations with various fatty acids. Glucose metabolites (G6P, F6P) are more negatively correlated with long-chain acyl-carnitines, hinting at a potential switch from glucose oxidation to fatty acid oxidation in diabetes (refer to Figure 4).

Table 1: The metabolite intensity trends across the groups (NC, DC, STD, LD, HD) for each lipid metabolite.

Groups (Intensity, cps)/Compounds, Q1/Q3, Da	Palmitic Acid (255.200/237.200)	Oleic Acid (279.200/263.200)	Linoleic Acid (279.200/261.200)	Arachidonic Acid (303.200/285.200)	Phosphatidylcholine (760.600/184.100)	Acyl Carnitine (C16) (400.000/85.000)	Acyl Carnitine (C18) (428.000/85.000)
NC	550	550	750	840	8800	5,000	3.7×10^4
DC	320	350	430	920	9000	3.5×10^5	8.5×10^4
STD	700	200	450	550	8340	2.9×10^5	6.0×10^4
LD	20	80	80	60	9940	1.7×10^5	5.0×10^4
HD	450	450	650	840	8200	2.25×10^4	8000

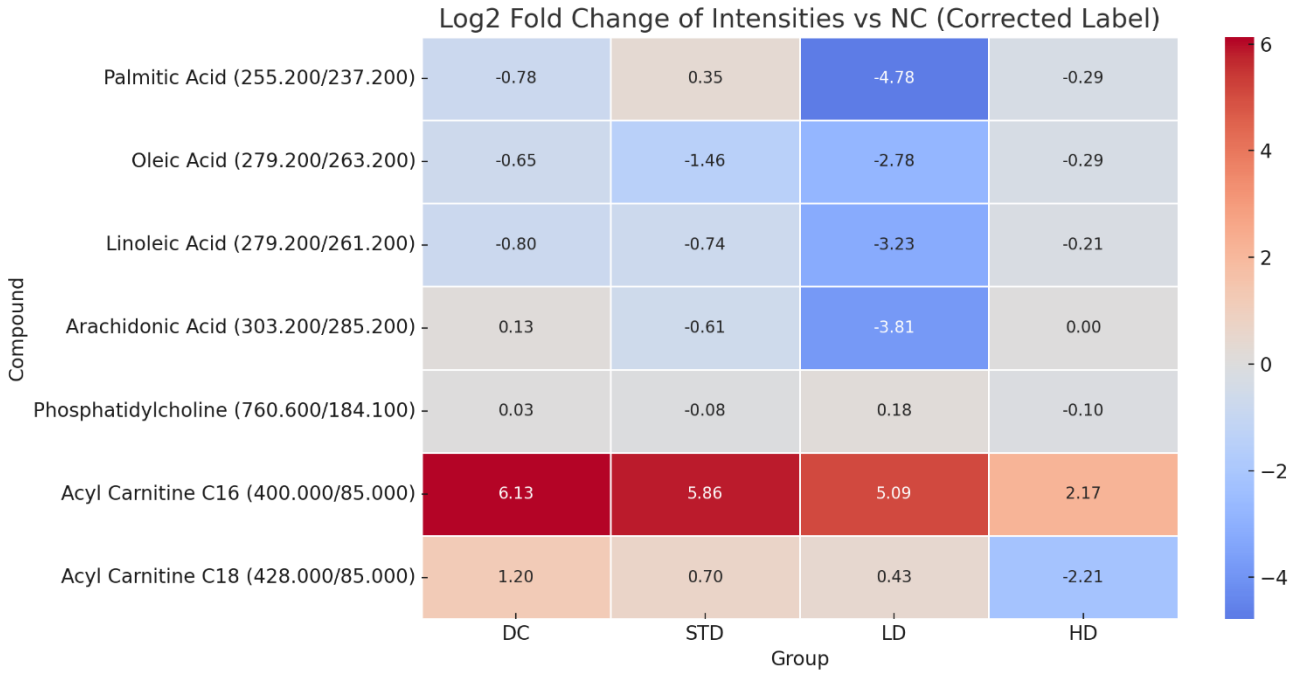

Figure 1: Log 2-Fold change of Intensities (cps) vs Normal Control.

Table 2: Correlation of Lipid Metabolites with T2DM in Animal Model.

Metabolite	Observed Trend	Biological Role	T2DM Relevance	Interpretation
Palmitic Acid (255.200/237.200)	↓ in DC; ↑ in STD, HD	Saturated FFA; energy source, lipotoxic	Depletion in tissue may reflect increased oxidation or impaired uptake.	HD dose partially restores balance, suggests improved lipid utilization.
Oleic Acid (279.200/263.200)	↓ in DC, LD; ↑ in HD	Monounsaturated FFA; membrane fluidity	Decrease may signal disrupted lipid remodeling or desaturation.	HD improves levels; recovery of lipid metabolism.
Linoleic Acid (279.200/261.200)	↓ in DC, LD; ↑ in HD	Polyunsaturated FFA; essential fatty acid	Altered levels reflect dietary/membrane remodeling defects.	HD shows best restoration toward NC profile.
Arachidonic Acid (303.200/285.200)	↑ in DC; normalized in HD	Inflammatory precursor (eicosanoids)	Elevated levels indicate inflammation.	HD reduces inflammation to control levels.
Phosphatidylcholine (760.600/184.100)	Stable; slight ↑ in LD	Major membrane phospholipid	Minor change indicates membrane lipid homeostasis is preserved.	Slight elevation in LD may suggest compensatory membrane turnover.
Acyl Carnitine C16 (400.000/85.000)	↑↑ in DC; ↓ in HD	Long-chain FA oxidation intermediate	Mitochondrial dysfunction marker	HD significantly reduces accumulation-improved β-oxidation.
Acyl Carnitine C18 (428.000/85.000)	↑ in DC; ↓ in HD	Reflects mitochondrial overload	Incomplete FA oxidation in insulin-resistant state.	HD normalizes levels-restores mitochondrial efficiency.

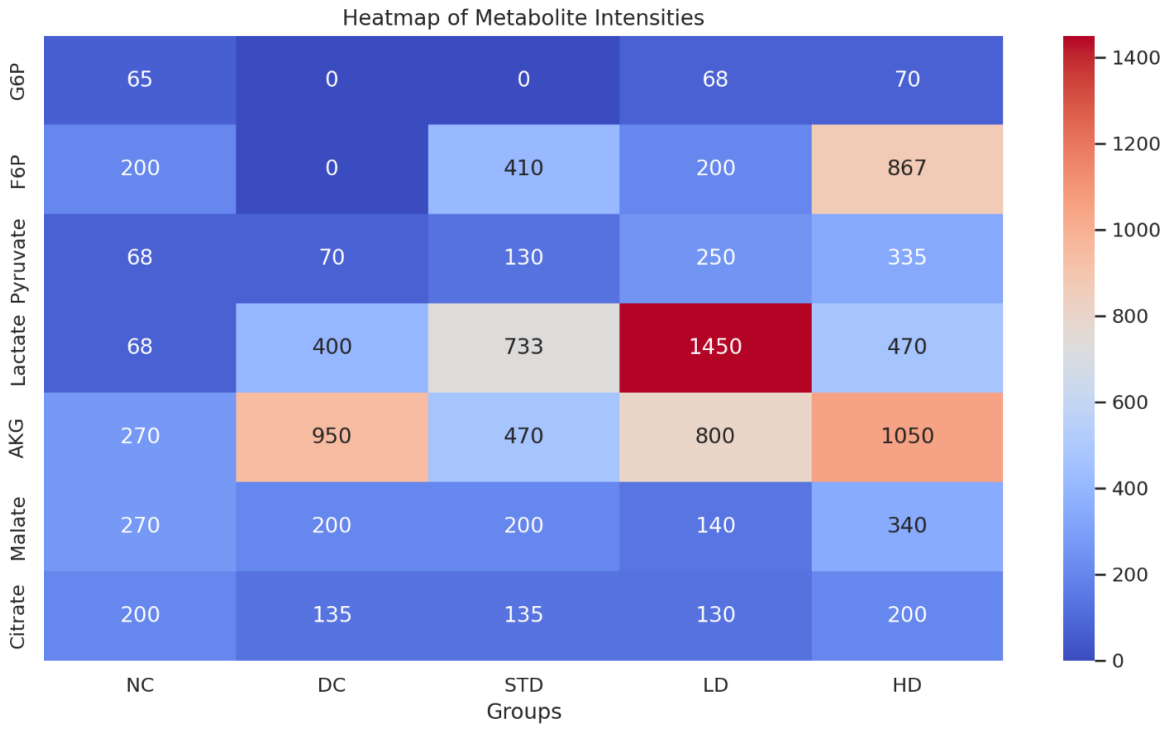

Figure 2: Heatmap of Glucose metabolite intensities.

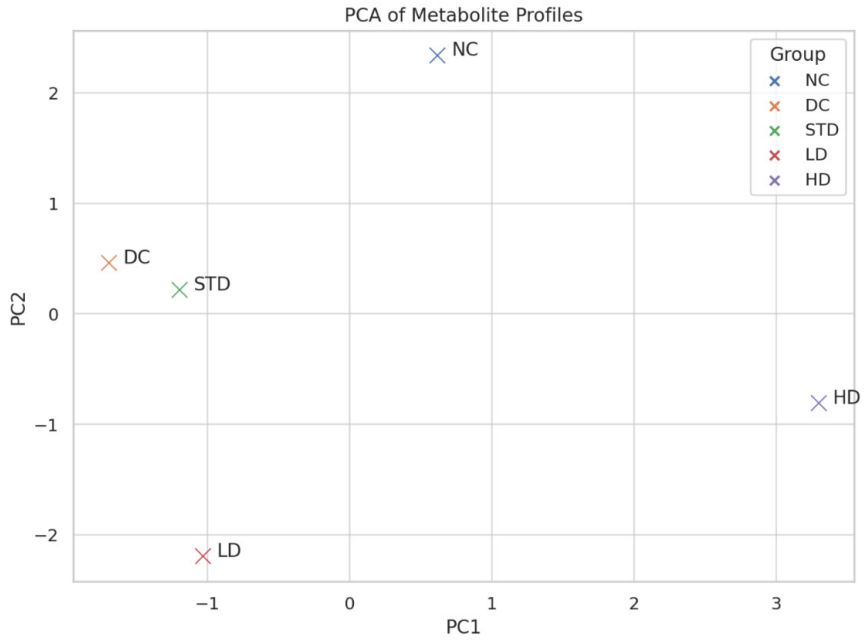

Figure 3: PCA of Glucose metabolites profiles: Highlights metabolic similarity/difference between groups.

The metabolite correlation network reveals key patterns of metabolic coordination: Blue edges: Positive correlations (when one metabolite increase, so does the other, r >0.7), Red edges: Negative correlations (when one metabolite increase, the other decreases, r <-0.7), Thicker lines indicate stronger relationships (Correlation coefficient closer to ±1). Oleic acid, linoleic acid, and arachidonic acid, which exhibit strong positive correlations with one another. This likely reflects shared biosynthetic or regulatory pathways, such as fatty acid elongation, desaturation, and possibly inflammatory lipid signaling. This lipid cluster is also positively correlated with TCA cycle intermediates, i.e. malate and citrate, suggesting a degree of lipid-TCA metabolic interplay. Another TCA and glucose metabolism, where malate, citrate, F6P, G6P, and pyruvate act as integrators. Malate and citrate emerge as

central nodes with connections to both carbohydrate-derived metabolites (e.g., G6P, F6P) and lipid metabolites, indicating that energy metabolism is a key driver of overall metabolic variation in the system. In contrast, certain metabolites exhibit strong negative associations. Lactate, in particular, shows robust negative correlations with citrate, malate, linoleic acid, oleic acid, and others. This suggests that elevated lactate levels reflective of anaerobic metabolism are inversely related to TCA cycle activity and lipid biosynthesis. Similarly, acyl-carnitines C16 and C18 display negative correlations - indicative of a metabolic shift toward fatty acid oxidation, potentially representing an adaptive response to impaired glucose metabolism such as in insulin resistance. Phosphatidylcholine appears less centrally connected but bridges both acyl-carnitines and TCA intermediates, hinting at a possible role in lipid transport or membrane remodeling during metabolic stress. Palmitic acid shows connections with both glucose-derived and lipid metabolites, suggesting it may serve as a metabolic interface between carbohydrate and lipid pathways (refer to Figure 5).

DISCUSSION

Studies in animal models often investigate how alterations in glucose and lipid metabolism affect overall metabolic health and disease. The study of how glucose metabolites (like pyruvate, lactate, and glycogen) and lipid metabolites (like fatty acids, triglycerides, and cholesterol) influence each other's pathways is a focus. Here we have examined how an animal's metabolism can shift between using glucose and fatty acids as fuel sources, depending on the physiological context. The accumulation of lipid breakdown intermediates in conditions such as excessive body fat contributes to the development of lipotoxicity, a key factor in metabolic disorders. According to the *Randle and colleagues*, increased fatty acid oxidation in the mitochondria produces metabolic signals that inhibit glucose utilization by suppressing key glycolytic enzymes.[15] This foundational concept of lipotoxicity has since evolved, with multiple mechanisms now recognized as contributors to impaired glucose metabolism.[16] In line with this understanding, the present study utilized LC-MS-based metabolomic profiling to examine key lipid and glucose metabolites in a rat model of T2DM. By identifying alterations in metabolites such as acylcarnitines, free fatty acids, and phospholipids, we aimed to uncover the biochemical shifts contributing to metabolic dysregulation. Advances in lipidomics have made it increasingly feasible to detect and annotate lipid signaling molecules from complex biological matrices, enabling a deeper understanding of inter-tissue communication and metabolic homeostasis.[17] The results from our model provide valuable insights into the systemic metabolic impact of the polyherbal formulation and support its role in modulating lipid signaling and glucose utilization pathways, thus potentially mitigating lipotoxic stress in T2DM. Monitoring changes in glucose metabolites can therefore serve as metabolic biomarkers to assess disease progression and the efficacy of therapeutic interventions. Restoring their balance is critical for improving insulin sensitivity, glucose utilization, and overall metabolic homeostasis in T2DM. Overall, the network structure underscores

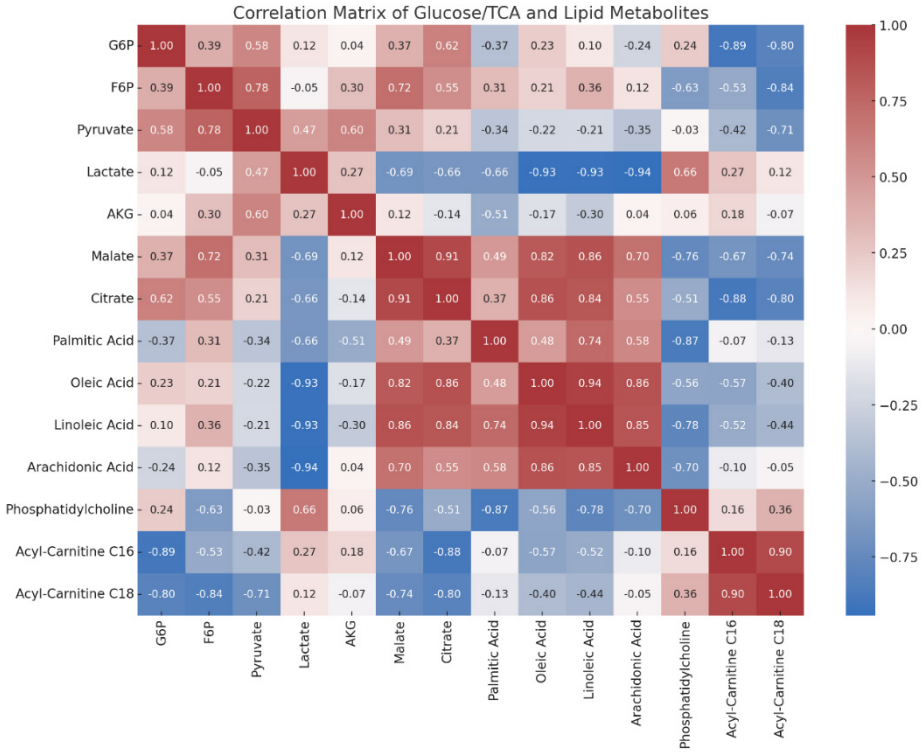

Figure 4: Correlation matrix between glucose/TCA metabolites and lipid metabolites.

Table 3: Glucose metabolites LC-MS intensity data with Q1/Q3 transitions (i.e., precursor/product masses in Da)

Groups (Intensity, cps)/ Compounds, Q1/Q3, Da	G6P (259.100/97.000)	F6P (259.100/79.000)	Pyruvate (87.000/42.800)	Lactate (88.900/42.800)	AKG (145.000/101.000)	Malate (133.000/115.000)	Citrate (190.800/57.000)
NC	65	200	68	68	270	270	200
DC	0	0	70	400	950	200	135
STD	0	410	130	733	470	200	135
LD	68	200	250	1450	800	140	130
HD	70	867	335	470	1050	340	200

Table 4: Glucose Metabolite-Level Interpretation.

Metabolite (Q1/Q3, Da)	Pathway	Trend Across Groups	Suggestions
G6P (259.1 / 97.0)	Glycolysis	↓↓↓ in DC, restored in HD	DC = glycolytic block; HD = recovered glucose phosphorylation.
F6P (259.1 / 79.0)	Glycolysis	↓↓↓ in DC, ↑↑ in HD	HD = improved glycolytic flux and PFK-1 activity.
Pyruvate (87.0 / 42.8)	Glycolysis end	↑ in all diabetic groups	Enhanced glycolysis or pyruvate overflow.
Lactate (88.9 / 42.8)	Anaerobic glycolysis	↑↑↑ in DC, LD, STD, HD	Shift to anaerobic metabolism; inefficient mitochondrial oxidation.
α-Ketoglutarate (145.0 / 101.0)	TCA cycle	↑↑ in DC, LD and HD	Mitochondrial overload
Malate (133.0 / 115.0)	TCA cycle	↓ in DC, STD and LD; ↑ in HD	HD restores downstream TCA cycle activity.
Citrate (190.8 / 57.0)	TCA cycle & lipid synthesis	↓ in DC, STD and LD; restored in HD	HD treatment revives citrate production = improved Acetyl-CoA entry into TCA.

Table 5: Correlation Glucose and TCA metabolites with lipid metabolites from the LC-MS data.

Group	Glycolysis (G6P, F6P)	Pyruvate	Lactate	TCA (Citrate, malate and AKG)	FFAs	PC	Acylcarnitines
NC	Normal	Normal	Low	Balanced	Normal	Normal	Normal
DC	Suppressed	Normal	High	High AKG, Low Citrate	↓ FFAs	Slightly ↑ PC	↑↑ Acyl-C16/18
STD	Partially Restored	Moderate	High	Moderate	Mixed	Normal	↑ Acyl-C16/18
LD	Partial Glycolysis	Moderately High	Very High	TCA Down	↓↓↓ FFAs	↑ PC	↑ Acyl-C16/18
HD	Restored	High	High	TCA Restored	Normal	Normal	↓ Acyl-C16/18

a tight regulation of unsaturated fatty acids, the centrality of TCA intermediates in metabolic flux, and the potential for metabolic inflexibility or reprogramming, particularly in scenarios where lactate and acyl-carnitines are upregulated. These patterns may provide insight into altered energy metabolism in pathological states such as type 2 diabetes.

T2DM impairs both glucose and lipid metabolism.[18] In this study, we evaluated the impact of a polyherbal formulation on glucose and lipid metabolites in a STZ-Nicotinamide-induced T2DM rat model, aiming to restore metabolic balance using traditional medicinal herbs. Our findings demonstrated that treatment with the polyherbal formulation, especially at the higher dose (500 mg/kg), led to: Restoration of key glycolytic intermediates such as G6P, F6P, pyruvate, and lactate. Normalization of TCA cycle metabolites, including malate, α-ketoglutarate, and citrate, reflecting improved mitochondrial function. Regulation of lipid metabolites such as palmitic, oleic, linoleic, and arachidonic acids, as well as acylcarnitines (C16 and C18) and phosphatidylcholine, suggesting improved lipid oxidation and reduced lipotoxic stress. These outcomes align with current understanding that metabolic resilience (TCA cycle, and fatty acid oxidation) is key to mitigating age-related diseases, particularly T2DM.[19] The improvement in

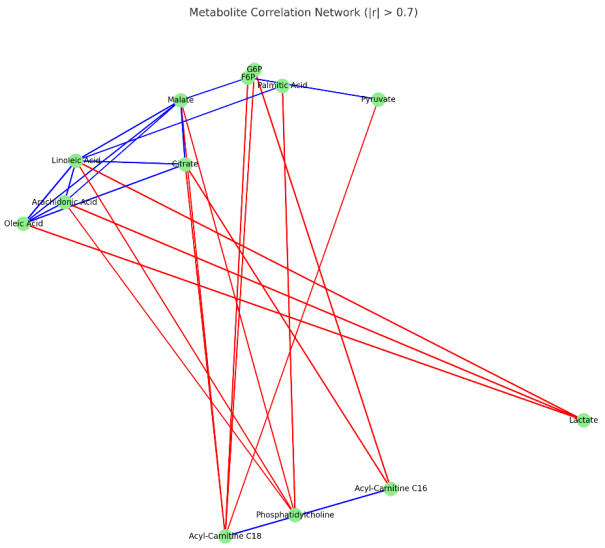

Figure 5: Metabolite correlation Network.

these pathways seen in our study may be indicative of enhanced mitochondrial function and energy efficiency. Importantly, the lipotoxicity hypothesis, first proposed by Randle *et al.*, explains how excess lipid metabolites impair glucose metabolism by blocking glycolytic enzymes.[20] Our LC-MS metabolomics approach revealed that the polyherbal formulation ameliorated this metabolic interference, highlighting its potential in reducing lipid-induced insulin resistance and restoring glucose utilization. Together, these results not only support the anti-diabetic efficacy of the polyherbal formulation but also underscore its potential to counteract age-related metabolic decline. By targeting multiple metabolic pathways simultaneously, the formulation may offer a multifaceted therapeutic approach to managing T2DM.

CONCLUSION

The polyherbal formulation significantly modulated the levels of these critical metabolites, suggesting restoration of glycolytic and TCA cycle flux, reduction of lipotoxic intermediates, and improved mitochondrial function. These findings support the potential of plant-based combinations in correcting metabolic imbalances associated with T2DM, offering a promising adjunct or alternative to conventional therapy.

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ABBREVIATIONS

G6P: Glucose-6-phosphate; **F6P:** Fructose-6-phosphate; **AKG:** Alpha-ketoglutarate; **TCA:** Tricarboxylic Acid cycle; **LC-MS:** Liquid Chromatography-Mass Spectrometry; **FFA:** Free Fatty Acids; **PC:** Phosphatidylcholine; **C16, C18:** Palmitoylcarnitine (C16), Stearoylcarnitine (C18); **PCA:** Principal Component Analysis; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **OECD:** Organisation for Economic Co-operation and Development; **EDTA:** Ethylenediaminetetraacetic Acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ETHICAL APPROVAL

The study was approved by the IAEC of Karnataka College of Pharmacy, under approval number KCP-IAEC/16/24-25/02/10/03/25.

SUMMARY

Metabolomic profiling using LC-MS revealed significant alterations in lipid and glucose metabolites in the type 2 diabetic preclinical model. The diabetic control group (STZ+NA) exhibited elevated acylcarnitines (C16, C18) and suppressed fatty acids, indicating mitochondrial dysfunction and impaired fatty acid oxidation. The standard drug, Glibenclamide (0.25 mg/kg, p.o.), showed partial correction, while the polyherbal formulation (250 mg/kg, p.o.) had minimal effect. Polyherbal formulation (500 mg/kg, p.o.) resulted in the most favorable outcome, with reduced acylcarnitines and recovery of fatty acid levels, suggesting improved mitochondrial activity and metabolic balance. Correlation analysis and PCA further supported group separation and identified metabolite relationships, while log2 fold change analysis highlighted key up- and downregulated metabolites. Overall, the polyherbal 500 mg/kg treatment demonstrated the strongest potential for reversing T2DM-related metabolic disturbances.

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