

Investigating the Multimodal Mechanism of a Polyherbal Anti-Diabetic Preparation Using 3T3-L1 adipocytes, C2C12 myotubes, and β TC-6 β -Cell Line Models

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ABSTRACT

Introduction: Hyperglycaemia is a complicated condition that includes reduced production of insulin, resistance towards insulin, and oxidative imbalance. Current therapies often have limited efficacy and adverse effects, prompting interest in safer, multitarget alternatives. Traditional polyherbal formulations have been used for diabetes, yet their mechanisms remain underexplored. **Objectives:** This study evaluated the antidiabetic potential of a standardized Polyherbal Preparation (EPHP 1) containing dried *Tinospora cordifolia* leaves, dried *Cinnamomum zeylanicum* bark, and dried *Trigonella foenum-graecum* and *Nigella sativa* seeds. **Materials and Methods:** Phytochemical standardization of ethanolic extracts performed. *In vitro* experiments included inhibition of α -glucosidase and α -amylase, glucose absorption in 3T3-L1 adipocytes and C2C12 myoblasts, and insulin secretion by stimulation of glucose in β TC-6 cells. The MTT test was used to evaluate cytotoxicity. Anti diabetic ability was determined in diabetic rats induced by streptozotocin. *In vivo* hepatic antioxidant parameters (SOD, CAT, GSH, MDA) was also evaluated. **Results:** EPHP 1 strongly inhibited α -glucosidase and α -amylase. In cells, it enhanced glucose uptake (up to 149% in 3T3-L1, 142% in C2C12) and increased insulin secretion (176% of control). *In vivo*, EPHP 1 (400 mg/kg) reduced blood glucose to 126.4 mg/dL by day 28 and restored antioxidant defenses, with no cytotoxicity observed. **Conclusion:** These findings indicate that EPHP 1 exerts potent antihyperglycemic effects via digestive enzyme inhibition, improved peripheral glucose uptake, enhanced insulin secretion, and attenuation of oxidative stress, providing scientific validation for its traditional use and supporting its potential as a safe, multitarget adjunct therapy for T2DM.

Keywords: Antidiabetic, *Cinnamomum zeylanicum*, Glucose Uptake, Insulin Secretion, *Nigella sativa*, Oxidative Stress, Polyherbal Formulation, *Tinospora cordifolia*, *Trigonella foenum-graecum*.

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INTRODUCTION

Hyperglycaemia is characterized by consistently elevated sugar levels in the blood, which can be caused by either reduced insulin action, abnormalities in insulin production, or a combination of the two. As microvascular and macrovascular problems grow over time, persistent hyperglycaemia adds to total morbidity and death.¹⁻³ Conventional pharmacotherapy for T2DM often targets a single mechanistic pathway (e.g., insulin secretion,

insulin sensitization, or inhibition of carbohydrate digestion).⁴ But problems like adverse effects, drug resistance, and limitations in the efficiency in complex disease states remain significant challenges. Because of this, supplementary or alternative therapy approaches-especially those based on medicinal plants-have garnered a lot of interest recently.^{5,6}

In traditional medicine systems (e.g., Ayurveda, Unani), polyherbal formulations are frequently used to treat multifactorial diseases, including diabetes.⁷ The rationale is that combining multiple botanical extracts can harness synergistic interactions among phytochemicals, thereby enhancing efficacy while potentially reducing adverse effects.⁸⁻¹⁰ Indeed, recent studies demonstrate that polyherbal formulations can affect a variety of biochemical and signalling mechanisms, viz., boosting insulin production, lowering insulin resistance, regulating glucose metabolism, and reducing oxidative stress and inflammation.¹¹



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This integrative, multitargeted approach may better address the complex pathophysiology of T2DM.¹²

Among botanicals with promising antidiabetic potential, *Tinospora cordifolia* (TC) (Menispermaceae) has received extensive interest. In animal and clinical models of diabetes, TC extracts have been demonstrated to reduce blood glucose, enhance lipid profiles, and boost antioxidant defenses. This is most likely due to modifications in insulin signaling, enzymes that break down carbohydrates, and free radical scavenging.^{13,14} The mechanistic basis also includes enhancement of glucose uptake in adipocytes and hepatocytes (e.g. TC stem extract stimulated uptake *in vitro*).¹⁵ as well as inhibition of α -amylase and α -glucosidase by its bioactive flavonoids, alkaloids, and terpenoids (e.g. magnoflorine) (e.g. Gupta *et al.* 2024).¹⁶ However, despite such evidence, its clinical translation has been limited, and the efficacy of TC in polyherbal combinations remains underexplored.

Other plant species with documented antidiabetic or insulinotropic effects include *Cinnamomum zeylanicum* (CZ), *Trigonella foenum-graecum* (TF), and *Nigella sativa* (NS). CZ has been reported to enhance insulin signaling and GLUT4 translocation.¹⁷ While TF (via trigonelline and saponins) can inhibit digestive enzymes and improve insulin sensitivity.¹⁸ and NS (via thymoquinone) exerts antioxidant and β -cell protective effects.¹⁹ When combined in a formulation, these botanicals may act via complementary and overlapping pathways, potentially producing additive or synergistic antidiabetic effects.

Despite some studies of individual herbs or two-component mixtures, there remains a scarcity of rigorous mechanistic evaluation of multi-component polyherbal formulations in both *in vitro* and *in vivo* settings. The lacuna in the literature emphasizes the need for comprehensive evaluation of phytochemical interactions, dose optimization, and mechanistic validation before translation to clinical use.^{20,21}

Thus, we attempted to determine the mechanism behind a standardized polyherbal preparation's antidiabetic efficacy in this work (named EPHP 1) comprising equal proportions of ethanol extracts of TC leaves, CZ bark, and seeds of TF, and NS which was proved to have hyperglycaemic potential in our previous publications.²²⁻²⁵ We sought to determine its impact on oxidative stress markers (CAT, MDA, GSH, SOD), its effect on uptake of glucose in C2C12 myoblasts and 3T3-L1 adipocytes, its insulin production in pancreatic β TC-6 cells, and its inhibitory activity on important enzymes for carbohydrate digestion. By integrating *in vitro* and *in vivo* mechanistic data, this work seeks to demonstrate robust evidence for the traditional applicability of such a polyherbal formulation and to assess its translational potential for adjunctive therapy in T2DM.

MATERIALS AND METHODS

Chemicals and Reagents

Every chemical and solvent utilized was of analytical quality. Ethanol (85% and 95% v/v), n-hexane, petroleum ether, Carboxymethyl Cellulose (CMC), nitric acid, and deionized water were obtained from Merck (India). Standard reference compounds (magnoflorine, cinnamaldehyde, trigonelline, and thymoquinone), enzymes (α -glucosidase from *S. cerevisiae*, α -amylase from pancreas of porcine), and substrates (p-nitrophenyl- α -D-glucopyranoside, 2-NBDG) were procured from SD Fine chem (India). Soluble starch purchased from HiMedia (India), and acarbose from Bayer (Germany). Differentiation agents (IBMX, dexamethasone, insulin), MTT reagent, DMSO, Triton X-100, and glibenclamide were obtained from Sigma-Aldrich. Antioxidant assays employed pyrogallol, DTNB, sodium lauryl sulfate, acetic acid, and thiobarbituric acid (all from Merck, India).

Collection and Authentication of Plant Materials

Dried bark of CZ, and dried seeds of TF and NS were procured from a local market in Kurnool, Andhra Pradesh. Dried leaves of TC were collected from their natural habitat in and around the Nallamala forest region, Srisailem, Kurnool District, Andhra Pradesh, India. Dr. K. Madhava Chetty, associate professor of botany at Sri Venkateswara University in Tirupathi, verified the authenticity of the plant materials by comparing them to specimens from herbariums. Identified specimens were deposited as voucher samples in the same laboratory for future reference.

Preparation of Extracts

Plant materials were extracted using ethanol, n-hexane and petroleum ether, and the antidiabetic potential of each extract was evaluated. Based on our earlier findings, ethanol extracts were selected for mechanistic studies, as they demonstrated the most potent antidiabetic activity.²²⁻²⁵

Quality control and Phytochemical characterization

The chemistry, manufacturing, and control parameters were used to standardize the extracts. Total ash, acid-insoluble ash, extractives soluble in water and 50% alcohol, loss on drying at 105°C, and the pH of a 1% w/w solution were among the physicochemical characteristics assessed. The heavy metals (mercury (Hg), arsenic (As), Lead (Pb) and Cadmium (Cd)) were directly analyzed using atomic absorption spectrophotometry after 0.25-5 mg of the extract was digested in a microwave system using concentrated nitric acid, diluted to 50 mL with deionized water, and 100 μ L spiked at 10 ppm concentration of internal standard. Phytochemical screening was also performed to identify major classes of compounds (alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids).

Development of Polyherbal preparation

Various combinations of the polyherbal preparation were formulated using ethanolic extract of CZ dried bark, TF dried seeds, NS dried seeds, and TC dry leaves in our previous research. Of them, the formulation with the code EPHP 1 containing equal proportions of all extracts showed notable antidiabetic effects when taken at 400 mg/kg body weight. Consequently, EPHP 1 was selected for further experimentation in the present study. The dose of 400 mg/kg was selected based on preliminary dose-ranging studies conducted in our laboratory (data not shown), where EPHP 1 was tested at 200, 400, and 800 mg/kg in STZ-induced diabetic rats. The 400 mg/kg dose consistently produced the most optimal glucose-lowering effect without any signs of toxicity, whereas 800 mg/kg did not provide additional benefit. Therefore, 400 mg/kg was chosen for mechanistic studies in the present work.

In vitro Enzyme Inhibition Assays

α -Glucosidase Inhibition

Using a spectrophotometric technique modified from Kim *et al.*, 2005.²⁶ The polyherbal preparation's inhibitory efficacy against α -glucosidase (from *Saccharomyces cerevisiae*, Sigma-Aldrich) was assessed. 250 μ L was the final volume in which the reaction was carried out. First, 50 μ L of the polyherbal preparation-which was made in a concentration range of 25-500 μ g/mL-was mixed with 100 μ L of 100 mM sodium phosphate buffer (pH 6.8) and 50 μ L of α -glucosidase solution (1.0 U/mL in phosphate buffer). After that, the mixture was preincubated at 37°C for 10 min. Following the preincubation phase, the enzymatic reaction was started by adding 50 μ L of 5 mM pNPG that had been dissolved in phosphate buffer. After that, the reaction mixture was incubated at 37°C for 20 min. The process was halted by adding 500 μ L of a 0.1 M sodium carbonate (Na_2CO_3) solution. Using a UV-vis spectrophotometer, the absorbance of the liberated p-nitrophenol was measured at 405 nm. As a typical positive control, acarbose was employed. A negative control was prepared using the solvent in place of the herbal preparation. To account for the intrinsic color of the polyherbal preparation, a corresponding blank was prepared for each concentration, containing all reagents except for the pNPG substrate. The absorbance of these blanks was subtracted from their respective test samples. The % inhibition was calculated using the following formula, and the extract concentration needed to block 50% of α -glucosidase Activity (IC_{50}) was plotted versus the log of the extract concentration. Every experiment was carried out in triplicate, and the mean $\pm$ Standard Deviation (SD) is used to represent the results.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance samples}}{\text{Absorbance of control}} \times 100$$

α -Amylase Inhibition

The polyherbal preparation's α -amylase inhibitory activity was assessed using a starch-iodine colorimetric technique, which

was slightly modified from Bernfeld's 1955 protocol.²⁷ Using a reaction mixture including 50 μ L of the polyherbal preparation at different dosages, namely 25-500 μ g/mL and 50 μ L of porcine pancreatic α -amylase (2.0 U/mL) in 20 mM sodium phosphate buffer (pH 6.9 with 6.0 mM NaCl), preincubation was carried out for 20 min at 37°C. 50 μ L of a 1% (w/v) soluble starch solution made in the same phosphate buffer was added to start the enzymatic reaction. The mixture was then incubated for 10 more min at 37°C. The reaction was halted by adding 100 μ L of 1.0 M Hydrochloric acid (HCl). 100 μ L of Lugol's solution (5 mM iodine in 5 mM potassium iodide) was added to the mixture in order to develop the color. The absorbance of the blue-black starch-iodine complex was measured at 565 nm using a UV-vis spectrophotometer. Acarbose served as the positive control and the solvent as the negative control. The inhibitory activity as a percentage of inhibition was calculated using the above formula.

Cytotoxicity Assay

Prior to treatment, cells were sown in 96-well plates and given time to adhere and differentiate: Over the course of 8-10 days, 3T3-L1 preadipocytes were planted at a density of 1.5×10^4 cells/well, cultivated to converge, and differentiated into adipocytes. C2C12 myoblasts were seeded and differentiated into myotubes over 5-7 days and maintained at 1.0×10^4 cells/well; β TC-6 cells were seeded at 8.0×10^3 cells/well. All seeding densities and differentiation protocols were optimized in preliminary experiments. Cells were subjected to progressive doses of the test extract (25-500 μ g/mL) for 24- and 48-hr following differentiation/attachment; a cytotoxic control (0.1% Triton X-100) and a solvent were also included. In a CO_2 incubator, the plates were incubated for three to 4 hr at 37°C. At the end of the treatment, each well received 20 μ L of MTT solution (5 mg/mL in phosphate-buffered saline, pH 7.3; final concentration: 0.5 mg/mL per well). To dissolve the formazan crystals, 100 μ L of DMSO was applied to each well after the supernatant was carefully removed. With a background correction at 630 nm, absorbance at 570 nm was measured using a microplate reader.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance samples}}{\text{Absorbance of control}} \times 100$$

The data are shown as mean $\pm$ SD. The experiments were conducted in duplicate wells and at least three different times. The dose-response curves were used to get the IC_{50} values.

Cell Culture Studies

Assay for glucose absorption in C2C12 myotubes and 3T3-L1 preadipocytes

The cellular uptake of glucose was quantitatively assessed using a fluorescently labeled glucose analog, 2-NBDG (2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxyglucose). To assess impact of experimental substances on glucose transport, the test was conducted on both differentiated 3T3-L1 adipocytes and C2C12 myotubes.

Cell Culture and Differentiation

In a humidified incubator, 3T3-L1 preadipocytes were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and high glucose at 37°C and 5% CO₂. Once the cells reached 100% confluence, they underwent differentiation into adult adipocytes. The cells were incubated for 48 hr using an adipogenic mixture consisting of 1 mM dexamethasone, 0.5 mM 3-Isobutyl-1-Methylxanthine (IBMX), and 1.7 mM insulin in DMEM with 10% Fetal Bovine Serum (FBS). After this induction phase, DMEM with 10% FBS and 1 µM insulin was added to the media for a further 48 hr. After ten days of differentiation, the cells were finally kept in DMEM with 10% FBS for four to six days, changing the media every two days, until they achieved morphological maturity with discernible lipid droplets.

C2C12 myoblasts were kept in high-glucose DMEM supplemented with 10% FBS at 37°C with 5% CO₂. To start the differentiation process, myoblasts at 80-90% confluence were moved to a differentiation medium made of DMEM enhanced with 2% horse serum. The medium was replaced every 24 hr until the cells fused to form multinucleated myotubes, which lasts for 7 days.

Assay Procedure

For the assay, differentiated cells (3T3-L1 adipocytes and C2C12 myotubes) in 96-well plates were starved by incubation in serum-free DMEM for 2-4 hr to minimize basal glucose uptake. The cells were starved and then treated for 24 hr with a vehicle control or several non-cytotoxic doses of polyherbal preparation (25-500 µg/mL) based on preliminary MTT tests. A positive control, insulin (100 nM), was included in all assays to ensure cell responsiveness. Following the course of treatment, the medium was aspirated, and the cells underwent two rounds of washing with Krebs-Ringer Bicarbonate Buffer (KRBB), which had been heated beforehand and included 2% Bovine Serum Albumin (BSA). After that, there were treated for 30 min at 37°C in KRBB-BSA containing 50 µM 2-NBDG. By washing the cells three times with ice-cold KRBB-BSA, unincorporated 2-NBDG was eliminated. The cells were then monitored using a microplate reader set to 485 nm for excitation and 535 nm for emission. Each condition was examined in triplicate, and the vehicle was applied to control cells. The relative fluorescence intensity in comparison to vehicle controls was used to quantify glucose absorption. The results are shown as mean±SD from a minimum of 3 separate trials.

Insulin secretion assay in β TC-6 pancreatic β -cells

Both baseline and stimulatory settings were used to evaluate the amount of insulin secreted by β TC-6 pancreatic β -cells in response to various secretagogues. The purpose of the experiment was to ascertain how the polyherbal formulation directly affected the release of insulin.

Cell Culture

We employed high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin-streptomycin, 2.5% Fetal Bovine Serum (FBS), 15% horse serum, and 2 mM L-glutamine to cultivate pancreatic β TC-6 cells. The cells were kept in a humidified incubator at 37°C with 5% CO₂. After the cells reached >90% confluence, they were quickly trypsinized, and the media was replaced every two to three days.

Assay Procedure

For the insulin secretion experiment, β TC-6 cells were planted in 24-well plates at a density of 1×10^4 cells/well, and they were allowed to adhere and recover for a full day. To create a low-glucose baseline condition, cells were preincubated for 2 hr at 37°C in Krebs-Ringer Bicarbonate Buffer (KRBB) containing 1.6 mM glucose before the experiment. The KRBB was taken out after this pre-incubation, and the cells were then incubated for 1 hr in new KRBB that contained either a high concentration of glucose (25 mM) or a baseline concentration of glucose (1.6 mM). To assess the effects of the polyherbal preparation, cells were co-incubated with various non-cytotoxic concentrations (25-500 µg/mL) determined from preliminary MTT assays alongside the high-glucose stimulus. A vehicle control (DMSO <0.1%) was included, and glibenclamide (10 µM) was used as a positive control in the assay.

Following the 1-hr incubation period, each well's supernatant was collected and kept at -20°C until analysis. Insulin in the supernatant is found using a mouse insulin ELISA kit. As per instructions of manufacturer, a commercial mouse insulin ELISA kit (Merck, USA) was used to measure the amount of insulin present in the culture supernatants. The insulinotropic effects of the substances under test were assessed using the insulin secretion data, which is reported as ng of insulin per µg of protein. A statistical analysis was conducted to ascertain the significance of the observed changes, and the findings were displayed as a fold change over the basal insulin secretion.

Evaluation of *in vivo* antidiabetic activity

Our previous studies demonstrated the antioxidant and antidiabetic potential of Polyherbal Preparations (PHPs) containing TC, CZ, TF, and NS. The extracts showed strong radical scavenging activity (DPPH and H₂O₂ assays), were safe up to 2000 mg/kg (OECD 420), and, in STZ diabetic rats, EPHP 1 at 400 mg/kg produced the most significant glucose-lowering effect, superior to other formulations and comparable to glibenclamide. These findings have been reported previously.²²⁻²⁵ In the present study, we build on this work by investigating the mechanistic action of EPHP 1 in cell culture models and evaluating *in vivo* antioxidant defenses through oxidative stress markers in pancreatic and hepatic tissues.

Evaluation of *in vivo* Antioxidant Evaluation

Oxidative stress parameters were assayed for EPHP 1 in liver tissue homogenates as described previously with minor modifications. Animals were sacrificed under anesthesia at the conclusion of the 28-day experiment, and the liver was removed, cleaned with ice-cold physiological saline, and processed right away to measure the oxidative stress markers Malondialdehyde (MDA), reduced Glutathione (GSH), Catalase (CAT), and Superoxide Dismutase (SOD).

Estimation of Superoxide Dismutase (SOD) Activity

The pyrogallol auto-oxidation technique was used to measure the activity of Superoxide Dismutase (SOD) in liver tissue.²⁸ Liver was removed immediately after sacrifice, washed in cold physiological saline, and then homogenized. After centrifuging the homogenate, the test was performed using the supernatant. 0.1 mL of newly made pyrogallol solution (2.5 mg/mL) and 2.9 mL of Tris-EDTA buffer (pH 8.5) were used in the reaction mixture. 10 μ L of liver supernatant were added for the test. The absorbance was measured at 90 and 120 sec using a UV-visible spectrophotometer at 420 nm. The quantity of enzyme needed to provide a 50% inhibition of pyrogallol auto-oxidation was referred to as one unit of SOD activity. The SOD activity units per milligram of protein were used to express the results.

Estimation of Catalase (CAT)

The dichromate-acetic acid technique was used to assess the amount of Catalase (CAT) activity in liver homogenates.²⁹ The reaction mixture, which included 0.1 mL of tissue homogenate, 0.5 mL of hydrogen peroxide substrate (20 mM H₂O₂), and 1.0 mL of phosphate buffer (0.01 M, pH 7.0), was incubated at room temperature. A 0.1 mL aliquot was transferred into 2.0 mL of dichromate-acetic acid reagent at 60-sec intervals to stop the reaction. After 10 min of heating the tubes in a boiling water bath, the amount of chromic acetate that resulted was determined using spectrophotometry at 570 nm. A standard curve was used to compute the residual H₂O₂, and catalase activity was represented as a first-order rate constant (K, min⁻¹) per milligram of protein (K/mg protein).

Estimation of Reduced Glutathione (GSH)

Ellman's reagent (DTNB, 5,5'-dithiobis (2-nitrobenzoic acid), 4 mg/mL in phosphate buffer, was used to spectrophotometrically measure the amount of reduced Glutathione (GSH) in liver homogenates.³⁰ 2.5 mL of sodium phosphate buffer (0.1 M, pH 8.0, including 1 mM EDTA) was combined with 30 250 μ L of homogenate. 50 μ L of Ellman's reagent (DTNB, 4 mg/mL) was added to start the reaction, and the mixture was then monitored at room temperature for 10 min. At an absorbance of 412 nm, the yellow colour that resulted from the production of 5-thio-2-nitrobenzoic acid (TNB) was quantified. A standard curve created using established GSH standards was used

to measure the GSH concentration, which was reported as micromoles per gram of tissue (μ mol/g tissue).

Estimation of Malondialdehyde (MDA)

Thiobarbituric Acid Reactive Substances (TBARS) were measured in liver homogenates to assess lipid peroxidation.³¹ A solution of 0.8% thiobarbituric acid, 1.5 mL of 20% acetic acid, 100 μ L of tissue homogenate, and 100 μ L of 8.1% sodium lauryl sulfate were combined for the test. Distilled water was used to get the final amount down to 4 mL. After being heated for 1 hr at 95 to 100°C in a boiling water bath, the mixture was cooled under running water and centrifuged for 10 min at 4000 rpm. At 532 nm, the supernatant's absorbance was measured. A molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ was used to compute the MDA concentration, which was then reported as nmol/g tissue.

Dose-Response Studies

Dose-response relationships for *in vitro* enzyme and cell-based assays were determined to quantify potency. Serial dilutions of EPHP 1 were tested in triplicate, and IC₅₀ or EC₅₀ values were calculated using nonlinear regression (4PL) with 95% confidence intervals. For cell-based assays, concentrations were selected based on preliminary cytotoxicity testing. *In vitro* dose-response was assessed using the two selected doses, and percent changes from baseline were analyzed to determine dose-dependent efficacy.

Statistical Analysis

All data are presented as mean $\pm$ Standard Error of the Mean (SEM) from at least three independent experiments ($n=3$ for *in vitro* assays; $n=6$ per group for *in vivo* studies). Statistical analysis was performed using One-Way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. Differences were considered statistically significant at $p<0.05$. All statistical calculations were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

The polyherbal preparation developed in this study has been used by humans, particularly in the villages of Kurnool District, Andhra Pradesh. The goal of our study was to establish scientific evidence for the folk lore medicine used by the villagers and to determine whether this polyherbal mixture has antidiabetic properties. In previous papers, we reported that this polyherbal preparation has potent anti-diabetic and hypolipidemic activity, which is linked to diabetes.²²⁻²⁵ As a result, we want to learn more about the mechanism underlying the polyherbal preparation's antidiabetic properties. In this context, we conducted experiments which will determine the mechanism underlying the antidiabetic property of the polyherbal preparation. α -Glucosidase and α -Amylase were evaluate the extract's capacity to control postprandial hyperglycemia. Similar to the mechanism of action of standards

like acarbose, the inhibition of these enzymes offers a viable technique for the control of type 2 diabetes.

To further understand the mechanism of action, cell-based experiments were conducted in addition to enzyme inhibition. Studies on glucose absorption were conducted using C2C12 myotubes and 3T3-L1 preadipocytes to assess how the polyherbal formulation affected peripheral glucose consumption. Since insulin resistance is characterized by reduced glucose uptake in muscle and adipose tissues, improvement in these pathways would suggest that the extract has insulin-sensitizing properties. Furthermore, insulin secretion was assessed in β TC-6 pancreatic β -cells to determine whether the preparation directly influences β -cell function and insulin release. When combined, these investigations enhance the enzyme inhibition tests and offer a more thorough mechanistic comprehension of the polyherbal preparation's antidiabetic effects.

Tissue antioxidant indicators such as GSH, SOD, MDA, GSH, and CAT were assessed in addition to these *in vitro* investigations. These biomarkers provide critical insight into the oxidative stress status and glucose metabolism in diabetes, thereby complementing the mechanistic data from *in vitro* assays and confirming the therapeutic efficacy of the preparation under physiological conditions.

Phytochemical screening, extract standardization and analytical characterization

One of the key objectives of our work was to carry out phytochemical screening and standardization of the Polyherbal Preparation (PHP). In our earlier published study (Venkatesh and Ramana, 2023), we confirmed the presence of four bioactive markers-magnoflorine, cinnamaldehyde, thymoquinone, and trigonelline-in the ethanolic extract of EPHP 1. Quantitative estimation using HPTLC revealed the following concentrations: magnoflorine 8.52 μ g/mL, cinnamaldehyde 7.62 μ g/mL, thymoquinone 9.21 μ g/mL, and trigonelline 8.22 μ g/mL. The method showed good linearity ($r^2=0.995-0.999$) with LOD and LOQ values ranging from 0.42-0.59 μ g/mL and 1.25-1.45 μ g/mL, respectively.²³ Alkaloids, flavonoids, phenolics, tannins, glycosides, and terpenoids were found by phytochemical screening, supporting the traditional usage of these plants to treat diabetes. While the antidiabetic efficacy of EPHP 1 has been reported earlier, the present study focuses on its mechanistic basis using *in vitro* cell-based assays and oxidative stress markers not previously described.²²⁻²⁵ The synergistic action of EPHP 1's markers may be responsible for its observed antidiabetic effect. Magnoflorine has been shown to inhibit α -glucosidase and modulate insulin-signaling pathways.³² Cinnamaldehyde improves glucose homeostasis by enhancing insulin signaling and GLUT4 translocation while reducing oxidative stress.^{33,34} Thymoquinone contributes to glycemic control through insulin-signaling activation, antioxidant

protection, and stimulation of insulin secretion.^{35,36} Trigonelline and its derivatives lower blood glucose by improving insulin signaling, suppressing carbohydrate-digesting enzymes, and reducing oxidative stress.^{37,38} These results collectively imply that EPHP 1's antihyperglycemic action involves both pancreatic and extra-pancreatic pathways. The results of various quality control parameters were shown in Table 1.

In vitro Enzyme Inhibition Assays

α -Glucosidase inhibition

EPHP 1 exhibited 12.5% inhibition of α -Glucosidase at 25 μ g/mL and 81.6% at 500 μ g/mL, respectively. Its IC_{50} was found to be 198.5 μ g/mL. The control drug acarbose showed 15.2% inhibition at 25 μ g/mL and 87.5% at 500 μ g/mL, with an IC_{50} value of 142.0 μ g/mL. The results were depicted in Figure 1.

α -Amylase inhibition

EPHP 1 exhibited 10.8% inhibition of α -amylase at 25 μ g/mL and 83.3 % at 500 μ g/mL, respectively. Its IC_{50} was found to be 212 μ g/mL. The control drug acarbose showed 13.4 % inhibition in α -amylase at 25 μ g/mL and 90.6 % at 500 μ g/mL, respectively. Its IC_{50} was found to be 156.5 μ g/mL. The results were depicted in Figure 1.

Cytotoxicity Evaluation (MTT Assay)

A range of EPHP 1 doses (25-500 μ g/mL) were administered to 3T3-L1 preadipocytes, C2C12 myoblasts, and β TC-6 cells, and their cytotoxic effects were evaluated. The EPHP 1 did not show any cytotoxic effects. The concentration of the EPHP 1 used, and the respective percent cell viability were tabulated and plotted in and Figure 2.

Cell Culture Studies

Although EPHP 1 remained below the benchmark positive controls, it clearly increased glucose absorption and insulin secretion in a dose-dependent manner. In 3T3-L1 and C2C12 cells, glucose uptake rose progressively from 110% at 25 μ g/mL

Table 1: Quality control parameters of EPHP 1.

Sl. No.	Parameters	Result
1.	Acid insoluble ash	2.563
2.	Loss on drying at 105	5.793
3.	pH	6.086
4.	Total ash	13.489
5.	Water soluble extractive	80.463
6.	50% alcohol soluble extractive	65.893
7.	Pb (mg/kg)	0.007
8.	Cd (mg/kg)	BDL
9.	Hg (mg/kg)	BDL
10.	As (mg/kg)	0.004

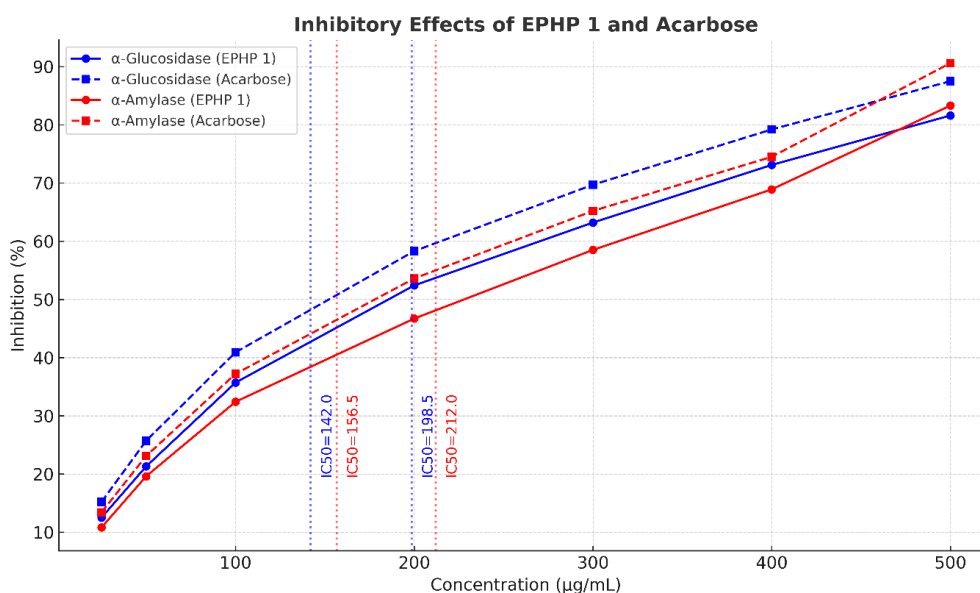


Figure 1: Dose-response inhibition of α -glucosidase (A) and α -amylase (B) by EPHP 1 and acarbose. Data are presented as mean $\pm$ SEM ($n=3$). IC₅₀ values were calculated by nonlinear regression (four-parameter logistic curve) using GraphPad Prism.

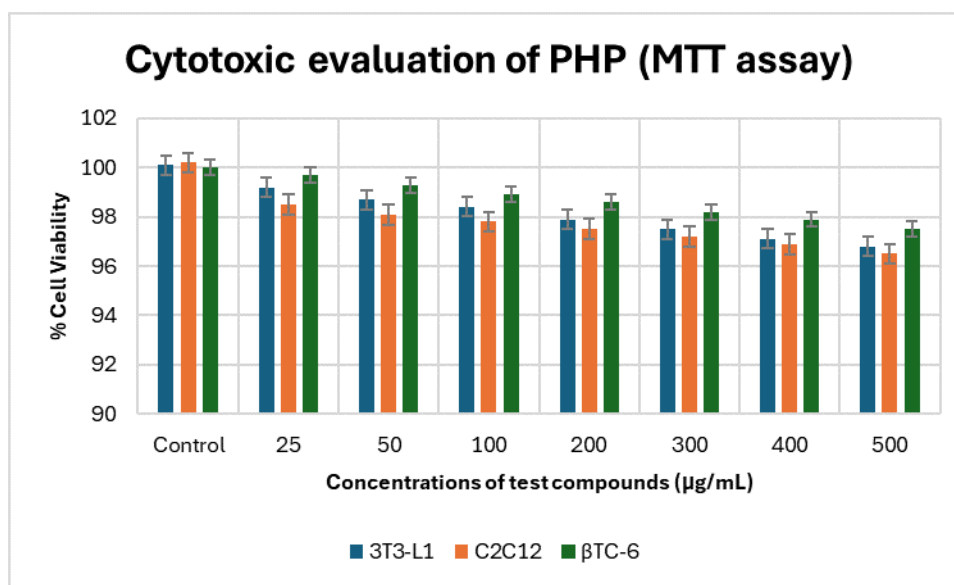


Figure 2: Effect of EPHP 1 on viability of 3T3-L1, C2C12, and β TC-6 cells after 24-hr exposure. Data are mean $\pm$ SEM ($n=3$). No significant cytotoxicity was observed at any concentration tested (one-way ANOVA, $p>0.05$ versus control).

to 149% at 500 μ g/mL (positive control: 152.3% and 145.7%, respectively). In β TC-6 cells, insulin secretion increased from 106% to 176% across the same concentration range (positive control: 182.5%). All test concentrations showed significant increases versus control ($p<0.05$). EC₅₀ values are included in the Table 2. These findings show that EPHP 1 enhances insulin production in pancreatic β -cells and increases glucose absorption in peripheral cells in a dose-dependent manner. Results of dose response effects of EPHP 1 on 3T3-L1 and C2C12 and β TC-6 Cells were given in Table 2 and Figure 3 shows representative bright-field images of 3T3-L1, C2C12, and β TC-6 cells following

EPHP 1 treatment. All three cell types remained viable, with no observable cytotoxic effects relative to control and positive treatments.

In vivo Antidiabetic Activity by STZ-induced diabetic rat model

The normal control group's blood glucose levels, which ranged from 76.68 $\pm$ 2.03 to 84.77 $\pm$ 2.33 mg/dL, stayed constant during the course of the research. On the other hand, following STZ treatment, the glucose levels of diabetic control mice significantly increased, reaching 313.73 $\pm$ 4.36 mg/dL on day 28. Over time,

Table 2: Dose-Dependent Effects of EPHP 1 on Glucose Uptake in 3T3-L1 and C2C12 Cells and Insulin Secretion in β TC-6 Cells.

Concentration ($\mu\text{g/mL}$)	Glucose uptake - 3T3-L1 (%)	Glucose uptake - C2C12 (%)	Insulin secretion - β TC-6 (%)
Control (0)	100 $\pm$ 1.2	100 $\pm$ 1.1	100 $\pm$ 1.1
Positive control	152.3 $\pm$ 2.1	145.7 $\pm$ 2.0	182.5 $\pm$ 1.8
25	110.4 $\pm$ 0.36 *	108.8 $\pm$ 0.38 *	106.0 $\pm$ 1.45 *
50	122.6 $\pm$ 0.42 *	120.9 $\pm$ 0.44 *	118.0 $\pm$ 1.53 *
100	134.8 $\pm$ 0.48 *	132.7 $\pm$ 0.51 *	132.5 $\pm$ 1.62 *
200	144.2 $\pm$ 0.55 *	139.1 $\pm$ 0.56 *	150.0 $\pm$ 1.71 *
300	146.3 $\pm$ 0.51 *	140.5 $\pm$ 0.50 *	160.0 $\pm$ 1.65 *
400	148.1 $\pm$ 0.49 *	141.3 $\pm$ 0.48 *	170.5 $\pm$ 1.59 *
500	149.4 $\pm$ 0.47 *	142.0 $\pm$ 0.46 *	176.0 $\pm$ 1.52 *
EC ₅₀ ($\mu\text{g/mL}$)	195.0	200.0	190.0

Values are mean $\pm$ SEM ($n=3$). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. * $p<0.05$ versus control; † $p<0.05$ versus positive control (insulin (100 nM) for 3T3-L1, C2C12) and glibenclamide (10 μM) for β TC-6 treatment).

glibenclamide (5 mg/kg) treatment successfully lowered blood glucose levels, which by day 28 had dropped to 121.36 $\pm$ 4.21 mg/dL. All EPHP-treated groups showed a dose-dependent antihyperglycemic effect. At 200 mg/kg, EPHP reduced glucose levels moderately, with values ranging from 167-179 mg/dL by day 28. Increasing the dose of EPHP 1 at 400 mg/kg resulted in a more pronounced reduction, with blood glucose decreasing to 126-144 mg/dL by day 28, comparable to the standard drug. These findings are consistent with our previous work and have been published in the literature, indicating that EPHP exhibits significant anti diabetic potential in STZ-induced diabetic rats, with higher doses showing enhanced efficacy Figure 4 shows the results of *in vivo* Antidiabetic potential of EPHP against induced diabetics.

In vivo Antioxidant Evaluation

Diabetic control animals showed marked reductions in SOD (37.65 $\pm$ 1.54 units/mg protein), CAT (64.89 $\pm$ 0.36 units/mg protein), and GSH (15.89 $\pm$ 0.10 $\mu\text{g/g}$ tissue), along with a substantial increase in MDA levels (61.94 $\pm$ 0.37 nmol/g tissue) compared to normal controls. Treatment with glibenclamide (5 mg/kg) restored these parameters close to normal values (SOD 84.77 $\pm$ 1.17, CAT 116.89 $\pm$ 1.09, GSH 40.63 $\pm$ 0.56, MDA 32.94 $\pm$ 0.56). Similarly, EPHP 1 at 400 mg/kg significantly improved antioxidant defenses (SOD 82.78 $\pm$ 0.56*, CAT 103.75 $\pm$ 0.90*, GSH 36.87 $\pm$ 0.93*) and reduced lipid peroxidation (MDA 38.90 $\pm$ 0.25*). These results are in line with our earlier research that was written up in the literature and shows that EPHP 1 successfully reduces oxidative stress in diabetic rats that have been given STZ. The outcomes of the *in vivo* Antioxidant Evaluation are shown in Table 3.

DISCUSSION

With the help of a polyherbal preparation (EPHP 1) that contains TC, CZ, TF, and NS, the current study sought to understand the mechanisms behind its antidiabetic potential. Our results show that EPHP 1 has a strong antidiabetic effect via a complex mechanism that includes inhibition of enzymes that break down carbohydrates, increased glucose uptake in peripheral tissues, stimulation of insulin secretion from pancreatic β -cells, and reduction of oxidative stress *in vivo*. All of these findings support the use of this herbal combination in ethnomedicine and demonstrate its potential as an additional treatment approach for Type 2 Diabetic Mellitus (T2DM).

Enzyme Inhibition and Postprandial Glycemic Control

Both α -amylase and α -glucosidase were shown to be concentration-dependently inhibited by EPHP 1, with IC₅₀ values of 198.5 $\mu\text{g/mL}$ and 212 $\mu\text{g/mL}$, respectively. The inhibitory effects were similar at higher dosages, while being somewhat less strong than acarbose (IC₅₀=142.0 $\mu\text{g/mL}$ for α -glucosidase; 156.5 $\mu\text{g/mL}$ for α -amylase) (Figure 1). At 500 $\mu\text{g/mL}$, EPHP 1 inhibited α -glucosidase by 81.6% and α -amylase by 83.3%, close to acarbose values (87.5% and 90.6%).

These findings suggest that EPHP 1 may effectively delay carbohydrate digestion and reduce postprandial hyperglycemia. Inhibition of these enzymes is a well-validated therapeutic approach in T2DM, as evidenced by the clinical success of acarbose and miglitol.³⁹ Moreover, several phytochemicals present in EPHP 1, such as magnoflorine, trigonelline, cinnamaldehyde, and thymoquinone, have been individually reported to inhibit these enzymes and regulate glucose absorption.^{40,41}

Effect of EPHP 1 on 3T3-L1, C2C12, and β TC-6 Cells viability

The MTT assay confirmed that EPHP 1 is non-cytotoxic to 3T3-L1, C2C12, and β TC-6 cells across the tested range (25-500 μ g/mL), with cell viability remaining above 96% in all cell lines ($96.8 \pm 1.2\%$, $96.5 \pm 1.4\%$, and $97.5 \pm 1.3\%$, respectively, at 500 μ g/mL; Figures 2, 3). This indicates that EPHP 1 does not impair cellular metabolism or proliferation, supporting its safety for *in vitro* and *in vivo* studies. The absence of cytotoxicity ensures that the observed increases in glucose uptake (up to 149.4% in 3T3-L1, 142% in C2C12) and insulin secretion (176% of control in β TC-6 cells) are due to pharmacological activity. These findings align with *in vitro* results, where EPHP 1 (400 mg/kg) reduced blood glucose to 126.4 mg/dL and restored antioxidant enzymes without adverse effects, confirming its safe multitarget antidiabetic potential.

Effects on Glucose Uptake in Peripheral Tissues

In a dose-dependent manner, EPHP markedly increased absorption of glucose by 3T3-L1 and C2C12 cells (Table 2). At

500 μ g/mL, glucose uptake rose to 149.4% in 3T3-L1 and 142.0% in C2C12 cells, nearly matching insulin (positive control: 152.3% and 145.7%). The calculated EC_{50} values (195 μ g/mL and 200 μ g/mL) indicate moderate potency.

This enhancement in glucose uptake indicates insulin-mimetic or insulin-sensitizing activity. Based on previous reports, cinnamaldehyde has been shown to enhance GLUT4 translocation and activate PI3K/Akt signalling (Anand *et al.*, 2010), while thymoquinone stimulates the AMPK pathway (Shaukat *et al.*, 2023). However, the present study did not directly investigate these molecular mechanisms.^{34,35} Thus, EPHP 1 appears to improve peripheral insulin sensitivity, addressing a central pathophysiological defect in T2DM.

Stimulation of Insulin Secretion

EPHP 1 enhanced glucose-stimulated insulin production in β TC-6 cells in a dose-dependent fashion (Table 2). At 500 μ g/mL, insulin secretion reached 176% of basal control, closely approaching the glibenclamide-induced response (182.5%). This finding suggests that EPHP 1 exerts a direct insulinotropic

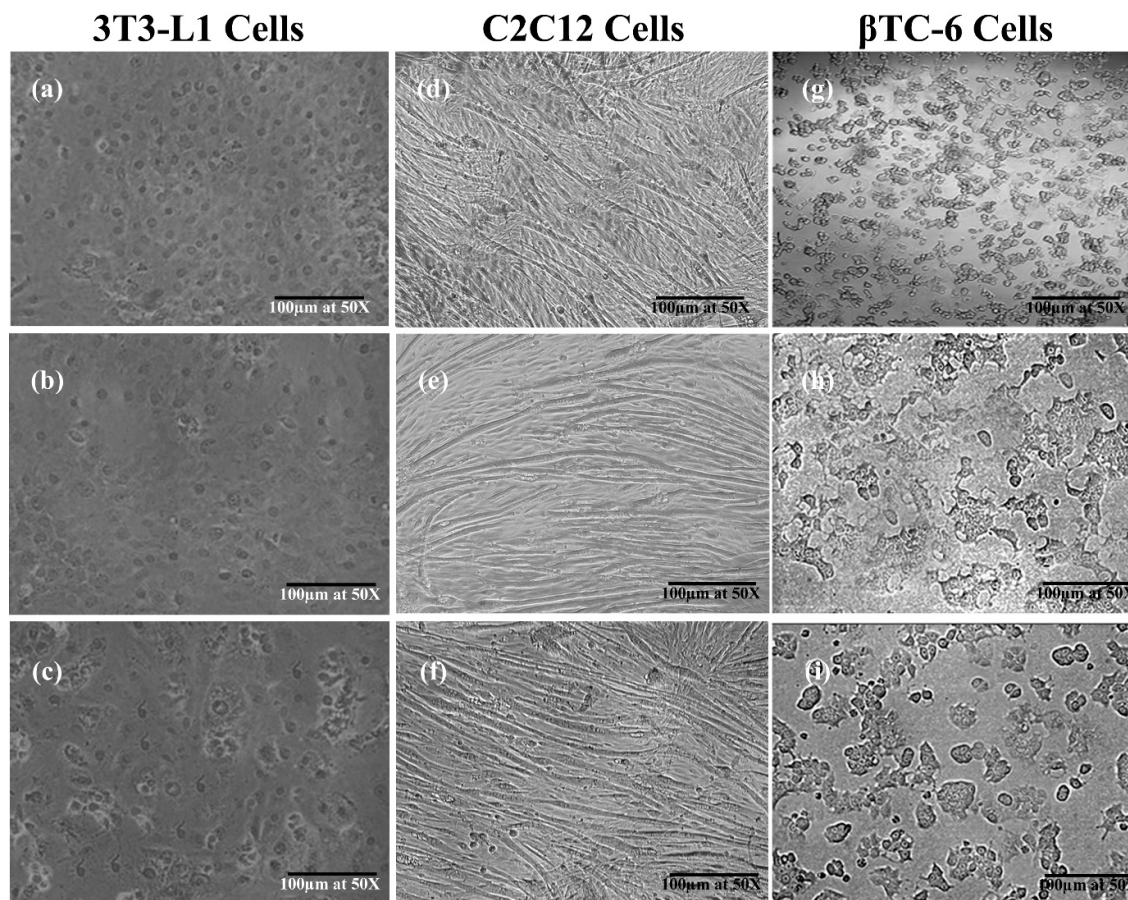


Figure 3: Representative images of 3T3-L1 adipocytes, C2C12 myotubes, and β TC-6 pancreatic β -cells captured using inverted light microscopy after 24-hr exposure to EPHP 1. Panels A, D, G: Untreated control cells of 3T3-L1, C2C12, and β TC-6, respectively; Panels B, E, H: Positive controls-insulin (100 nM) for 3T3-L1 and C2C12 cells, and glibenclamide (10 μ M) for β TC-6 cells; Panels C, F, I: Cells treated with EPHP 1 at 500 μ g/mL, showing that all three cell types remained viable with no observable cytotoxicity compared with controls. All images were acquired at 50 $\times$ magnification under bright-field conditions using an inverted microscope. Scale bars correspond to 100 μ m.

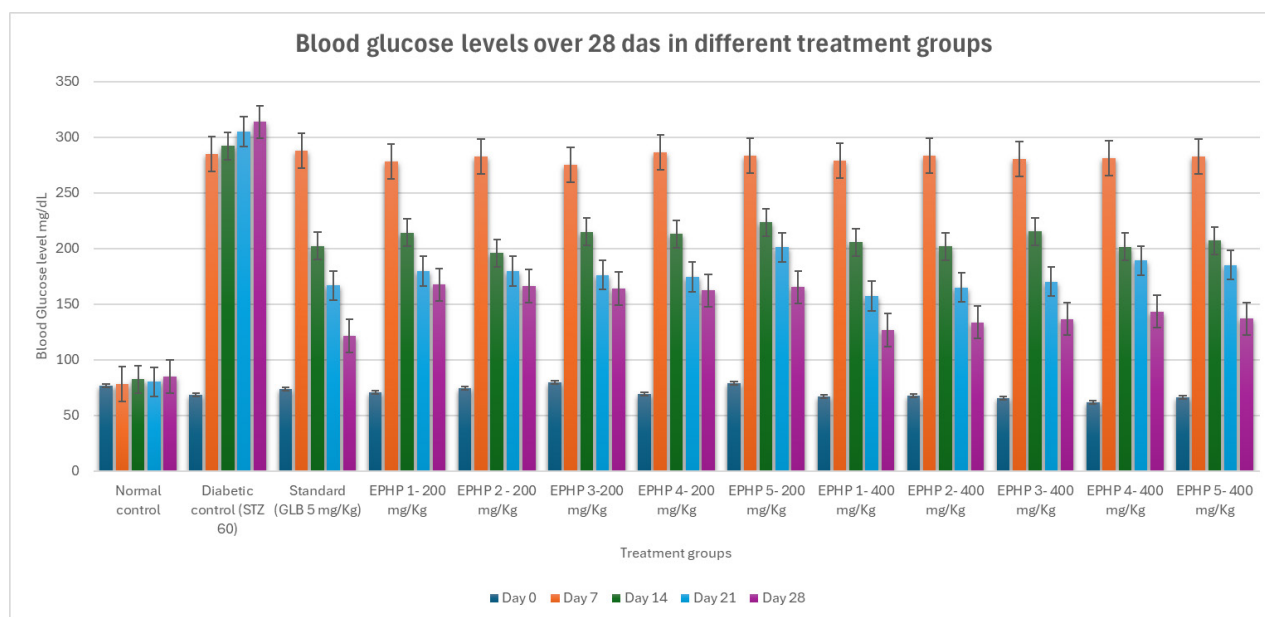


Figure 4: Effect of EPHP 1 on fasting blood glucose levels in STZ-induced diabetic rats over 28 days. Data are mean $\pm$ SEM ($n=6$ per group). ** $p<0.01$ versus diabetic control; *** $p<0.001$ versus diabetic control (one-way ANOVA followed by Tukey's *post hoc* test).

effect, potentially through β -cell stimulation or protection against oxidative stress.

Such dual action-enhancing both insulin release and peripheral glucose uptake-confers a therapeutic advantage, since most synthetic antidiabetic agents act via a single mechanism. Further more, as show in Figure 3 that *3T3-L1 adipocytes*, *C2C12 myotubes*, and *β TC-6 pancreatic β -cells* maintained normal morphology after 24-hr exposure to EPHP 1 at 500 μ g/mL. No signs of cytotoxicity, such as cell shrinkage or detachment, were observed compared with untreated controls or positive controls (insulin or glibenclamide). This confirms that EPHP 1 is well tolerated by these cell types at the tested concentration. The preserved cellular integrity supports the reliability of subsequent functional assays, including glucose uptake and insulin secretion. Overall, the images provide visual evidence that EPHP 1 is non-toxic and safe for *in vitro* evaluation. Similar insulinotropic effects have been reported for NS and TF extracts in β -cell models.⁴²⁻⁴⁴

In vitro Antihyperglycemic Efficacy

In STZ-induced diabetic rats, EPHP 1 (400 mg/kg) significantly lowered blood glucose from 278.8 mg/dL (day 7) to 126.4 mg/dL (day 28) (Figure 4), closely resembling the standard glibenclamide group (121.3 mg/dL). Lower doses (200 mg/kg) produced moderate reductions, highlighting a clear dose-response relationship. These results confirm that the *in vitro* observations translate into systemic antihyperglycemic effects *in vivo*.

Recent studies of polyherbal formulations containing cinnamon, fenugreek, and other botanicals have reported similar glucose-lowering effects in diabetic rodent models and even in prediabetic human subjects, lending strong external validation to our findings.^{45,46}

Antioxidant Defense and Oxidative Stress Reduction

Diabetic control rats exhibited oxidative imbalance, with reduced SOD (37.65 U/mg), CAT (64.89 U/mg), and GSH (15.89 μ g/g), along with elevated MDA (61.94 nmol/g). Treatment with EPHP 1 (400 mg/kg) significantly restored antioxidant markers (SOD: 82.78 U/mg, CAT: 103.75 U/mg, GSH: 36.87 μ g/g) and reduced lipid peroxidation (MDA: 38.9 nmol/g) (Table 3). These values were comparable to glibenclamide treatment, indicating potent antioxidant activity.

Oxidative stress is a critical factor in β -cell dysfunction and progression of diabetic complications. By restoring antioxidant enzyme activities, EPHP 1 not only improves glycemic control but may also provide protection against long-term diabetic complications.

These *in vivo* observations align with recent reviews emphasizing the antioxidant mechanisms of plant-based foods in diabetes management.⁴⁷

Significance and Mechanistic Insights

The combined results indicate that EPHP 1 acts through both pancreatic and extra-pancreatic pathways. Postprandial hyperglycemia is addressed by inhibiting digestive enzymes, and better glycemic control is facilitated by increased insulin production and glucose absorption. Additionally, the strong antioxidant properties mitigate oxidative stress, which is pivotal in slowing diabetes progression and complications. Such multitarget activity is consistent with the holistic therapeutic approach in traditional medicine, where combinations of botanicals are used to achieve synergistic efficacy.

Table 3: Impact of EPHP 1 Treatment at 400 mg/kg body weight on Hepatic Antioxidant Status and Oxidative Stress in Diabetic Rats.

Group	Treatment	SOD (units/mg liver protein)	CAT (units/mg liver protein)	GSH ($\mu\text{g/g}$ tissue)	MDA (nmol/g tissue)
Normal control	Vehicle (normal saline)	97.12 $\pm$ 1.903	132.86 $\pm$ 0.563	44.99 $\pm$ 0.254	30.65 $\pm$ 0.462
Diabetic control	STZ 60 mg/kg	37.65 $\pm$ 1.536	64.89 $\pm$ 0.362	15.89 $\pm$ 0.101	61.94 $\pm$ 0.373
Glibenclamide (5 mg/kg)	STZ 60 mg/kg + Glibenclamide 5 mg/kg, i.p	84.77 $\pm$ 1.174	116.89 $\pm$ 1.093	40.63 $\pm$ 0.563	32.94 $\pm$ 0.562
EPHP 1 (400 mg/kg)	STZ 60 mg/kg + EPHP 1 400 mg/kg	82.78 $\pm$ 0.563*	103.75 $\pm$ 0.901*	36.87 $\pm$ 0.932*	38.90 $\pm$ 0.254*

* Values are expressed as mean $\pm$ SEM ($n=6$ per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. $p<0.05$ versus the diabetic control group.

FINDINGS AND LIMITATIONS

While EPHP 1 exhibited strong efficacy across multiple models, its IC_{50} values for enzyme inhibition were slightly higher than acarbose, suggesting moderate potency in this pathway compared to the synthetic drug. However, this limitation is counterbalanced by the broader mechanistic effects observed in cell and animal models. Another limitation is that this study was restricted to short-term *in vivo* evaluation in STZ-induced diabetic rats, which may not fully mimic the chronic and progressive nature of human T2DM. Furthermore, the absence of detailed molecular studies (e.g., signaling pathway assays, gene expression profiling) limits mechanistic precision.

FUTURE DIRECTIONS AND APPLICATIONS

The present findings justify further exploration of EPHP 1 in long-term diabetes models and clinical studies to validate safety and effectiveness in people. Further mechanistic understanding may be obtained by molecular investigations examining the role of the PI3K/Akt, AMPK, and GLUT4 signaling pathways. Because EPHP 1 affects both insulin secretion and sensitivity, it might be used as a supplement to traditional antidiabetic medications, lowering dosages and decreasing side effects. The notable antioxidant effects further highlight its potential to reduce the risk of diabetes-induced complications, including cardiovascular, neurological, and renal impairments. Additionally, the present study did not determine enzyme kinetic parameters such as K_m , V_{max} , or the mode of inhibition (competitive/non-competitive) for EPHP 1 against α -glucosidase and α -amylase. Future studies employing Lineweaver-Burk plots are needed to fully characterize the inhibitory mechanism.

CONCLUSION

Collectively, the findings suggest that EPHP 1 exerts multitarget antidiabetic activity by modulating carbohydrate metabolism, improving peripheral insulin sensitivity, stimulating insulin

secretion, and enhancing antioxidant defenses. This holistic mechanism reflects the traditional rationale for polyherbal formulations and supports their potential as adjunctive therapy in T2DM. However, long-term safety, clinical validation, and molecular mechanistic studies are warranted to confirm translational utility.

ACKNOWLEDGEMENT

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ABBREVIATIONS

CZ: *Cinnamomum zeylanicum*; **NS:** *Nigella sativa*; **TC:** *Tinospora cordifolia*; **TF:** *Trigonella foenum-graecum*; **As:** Arsenic; **CAT:** Catalase; **Cd:** Cadmium; **CMC:** Carboxymethyl Cellulose; **DMSO:** Dimethyl Sulfoxide; **DTNB:** 5,5'-Dithiobis (2-nitrobenzoic acid); **EDTA:** Ethylenediaminetetraacetic Acid; **GSH:** Reduced Glutathione; **HCl:** Hydrochloric Acid; **Hg:** Mercury; **IBMX:** 3-Isobutyl-1-methylxanthine; **MDA:** Malondialdehyde; **Na₂CO₃:** Sodium Carbonate; **NaCl:** Sodium Chloride; **Pb:** Lead; **SOD:** Superoxide Dismutase; **TNB:** 5-Thio-2-nitrobenzoic Acid; **TBARS:** Thiobarbituric Acid Reactive Substances; **DMEM:** Dulbecco's Modified Eagle Medium; **KRBB:** Krebs-Ringer Bicarbonate Buffer; **BCS:** Bovine Calf Serum; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **ELISA:** Enzyme-Linked Immunosorbent Assay; **EPHP 1:** Experimental Polyherbal Preparation 1; **FBS:** Fetal Bovine Serum; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; **PHPs:** Polyherbal Preparations; **STZ:** Streptozotocin; **UV-vis:** Ultraviolet-visible Spectrophotometer; **i.p:** Intraperitoneal; **SD:** Standard Deviation; **T2DM:** Type 2 Diabetes Mellitus.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study investigated the antidiabetic potential and underlying mechanisms of a standardized polyherbal preparation (EHP 1) composed of *Tinospora cordifolia*, *Cinnamomum zeylanicum*, *Trigonella foenum-graecum*, and *Nigella sativa*. Phytochemical analyses confirmed the presence of bioactive compounds such as magnoflorine, cinnamaldehyde, trigonelline, and thymoquinone.

EHP 1 significantly inhibited carbohydrate-digesting enzymes (α -glucosidase, IC_{50} =198.5 μ g/mL; α -amylase, IC_{50} =212 μ g/mL), thereby supporting postprandial glucose regulation. In cell-based assays, it enhanced glucose uptake in 3T3-L1 adipocytes (up to 149%) and C2C12 myotubes (142%), while also stimulating insulin secretion in β TC-6 cells (176% of control), without cytotoxicity. In an *in vivo* study using streptozotocin-induced diabetic rats, EHP 1 (400 mg/kg) lowered blood glucose to near-normal levels (126 mg/dL by day 28) and restored hepatic antioxidant defenses (SOD, CAT, GSH) while reducing lipid peroxidation (MDA). These effects were comparable to glibenclamide.

The findings highlight that EHP 1 exerts multitargeted antihyperglycemic action through digestive enzyme inhibition, enhanced insulin secretion, improved peripheral glucose uptake, and attenuation of oxidative stress. This provides scientific validation for its traditional use and supports its potential as a safe, complementary therapy for Type 2 Diabetes Mellitus (T2DM).

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