MICROARCHITECTURE OF PANCREATIC ISLET AND ITS MODULATION IN DIABETIC ANIMAL DUE TO HERBAL DRUG THERAPY

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disorder that has reached pandemic levels globally, affecting millions at an alarming rate and contributing to significant morbidity and mortality. The disease is characterized by persistent hyperglycemia resulting from insulin resistance and/or a progressive loss of pancreatic β-cell function. Islets of Langerhans, a highly vascularized micro-organ of the pancreas, are scattered throughout the exocrine acinus and account for approximately 1–2% of the total pancreatic mass. These consist of 15–20% α-cells (glucagon-producing), 60–65% β-cells (insulin-producing), and the rest are polypeptide P.P. cells and δ cells. Maintaining an optimal islet size is crucial for enhanced functionality. The mantle-core architecture of the cell subtypes within the islets enhances insulin secretion via a paracrine effect. Normal insulin secretion from pancreatic β-cells is facilitated by calcium influx and membrane depolarization.

Motor proteins have garnered significant interest in this field because of their active role in membrane and organelle trafficking. Among the myriad motor proteins, myosin Va stands out for its functions in mRNA trafficking, exocytosis of secretory vesicles, and organelle transport in neuroendocrine cells. This motor protein is highly expressed in endocrine organs and is directly involved in the localization and secretion of endocrine granules. By reviewing these characteristic features of myosin Va, the present study examined the differential expression patterns of the myosin Va motor protein in the endocrine region of diabetic and herbal drug-treated animals.

Despite the availability of various pharmacological treatments, including biguanides, sulfonylureas, thiazolidinediones, and GLP-1 receptor agonists, the long-term use of these synthetic drugs often results in adverse side effects, such as cardiovascular risk (associated with sulfonylureas and thiazolidinediones), gastrointestinal complications, and liver and kidney dysfunctions (associated with biguanides and GLP-1 receptor agonists). These side effects can significantly impact daily activities. Consequently, interest in alternative therapies, particularly those based on natural products and traditional medicine systems, which offer the potential for minimal side effects and have been used for centuries in various cultures to manage chronic health conditions, including diabetes, is increasing.

Anecdotal evidence suggests that ethnopharmacological agents may have a favourable impact when used as adjunct therapies for managing various ailments, including diabetes. Recent WHO reports indicate that many developing countries (70–80%) rely on traditional methods of treatment involving the use of medicinal plants to manage various

chronic health conditions, including diabetes, due to their lower risk of side effects than modern therapies.

Current research focuses on identifying potent ethnopharmacological agents with promising biological activity. Natural products, especially those derived from medicinal plants, have shown potential antidiabetic properties through various mechanisms, including enhancing insulin secretion, improving insulin sensitivity, inhibiting carbohydrate-digesting enzymes, and protecting pancreatic β-cells from oxidative stress.

This study aims to validate the antidiabetic efficacy of polyherbal formulations and individual plant extracts, including *Tinospora cordifolia* and *Mangifera indica*, through a multitiered approach involving *in silico*, *in vitro*, and *in vivo* antidiabetic models. It also explores the microarchitectural changes in pancreatic islets in diabetic animal models and their modulation with herbal drug therapy. Additionally, we assessed the potential restoration of pancreatic β-cell function and the expression of the myosin Va motor protein following treatment with these natural products. Furthermore, the present study also examined the active role of myosin Va in the transportation of pancreatic β-cells toward the final stage of exocytosis.

The primary objective of the present research was to evaluate the antidiabetic efficacy of the developed polyherbal formulation by utilizing *in silico*, *in vitro,* and *in vivo* antidiabetic models. To investigate the effects of standardized ayurvedic formulations (prepared from selected plants) on the morphology of the pancreatic islets in diabetic rodents. To study the secretion capacity of endocrine cells on the basis of the intensity of the Myosin Va motor protein after treatment with standardized formulations.

Materials and Methods:

The sixteen selected plant parts were freshly collected from the flora of Gandhamardhan Hill, Bargarh, Odisha, and validated by the Taxonomist Regional Plant Research Centre (RPRC) Bhubaneswar. After drying in a tray dryer, the samples were powdered with a versatile pulverizer and passed through a sieve of 10 mesh for uniformity. The polyherbal formulation was prepared by adding all sixteen plants in equal proportions and subjected to aqueous extraction (APE) with a Soxhlet apparatus. After standardization, three plants (*T. cordifolia*, *S. cumini* & *M. indica*) were further used to prepare another aqueous polyherbal formulation by mixing in equal proportions and then subjected to aqueous extraction (APF) via a Soxhlet apparatus. For individual preparations (*T. cordifolia* & *M. indica*) of plant extracts, the powdered plants were subjected to hydroalcoholic extraction (ethanol:water) via a microwave extraction system. After extraction, the yield extract was lyophilized and used in the present study.

Quantitative and biochemical analysis

The lyophilized extracts (APE, APF, *T.C., M.I.*) were quantified for identification of the bioactive compounds via advanced quantitative techniques such as Fourier transform infrared spectroscopy (FTIR), ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS), and Gas chromatography–mass spectrometry (GC–MS). The total phenolic content (TPC) was quantified using gallic acid equivalent (GAE), and the total flavonoid content (TFC) was quantified using quercetin equivalent (Q.E.). All the extracts were subjected to biochemical analyses, including free radical scavenging activity (DPPH, ABTS, and FRAP) and *in vitro* enzyme inhibition assays (such as α-amylase, α-glucosidase, and β-glucosidase). The results were expressed as IC_{50} values to determine the potency of the extracts.

In silico study

The identified bioactive compounds from the analytical studies of the extracts were prepared. Insulin-like growth factor-I (IGF-I) and glucose transporter 4 (GLUT 4) proteins were selected for docking studies against the selected bioactive compounds. The Schrodinger molecular environment was utilized for the docking and prediction of the ADME properties of these proteins and ligands. This analysis helped to elucidate potential mechanisms of action for the phytochemicals.

In vitro cell assays

The *in vitro* cell assays were performed using 3T3L1 (adipocytes) and MIN6 β (isolated from pancreatic islets) cell lines. The cell cytotoxicity assay was performed using the 3T3-L1 cell line. A cellular glucose uptake assay utilizing the 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG) fluoroprobe was performed in the MIN6 β cell line. This assay was performed to evaluate the effects of the extracts on insulin-mediated glucose uptake. The 3T3L1 adipocyte line was used for the lipid accumulation study. The oil red O staining technique was used to detect the accumulated lipid droplets.

In vivo antidiabetic study

Wistar rats were selected for the present antidiabetic study. Acute and subacute oral toxicity studies were performed prior to the experiment to determine the safe dose. Alloxan was injected intraperitoneally *(i.p.)* for the development of diabetic models. The rats were randomly divided into control, untreated diabetic control, positive control, and herbal drug-treated groups. Diabetic animals were treated with either metformin or herbal extracts for four weeks. Blood glucose and HbA1c levels were measured to assess long-term glucose control. Serum markers, including glucose, albumin, urea, creatinine, cholesterol,

triglycerides, and liver enzymes (SGPT, SGOT, ALP), were evaluated to assess the safety and efficacy of the treatment. Upon completion of the experiment, the animals were sacrificed by mild anaesthesia (isoflurane), blood was collected via cardiac puncture for biochemical parameter studies, and the pancreatic tissues were fixed in 10% neutral buffered formalin (NBF). The fixed tissues were processed, and paraffin blocks were prepared. Each block was subjected to H&E and IHC staining with primary antibodies, such as anti-synaptophysin, anti-insulin, anti-glucagon, and anti-myosin. The stained slides were observed under a microscope in the bright field region and quantified using Image J software.

Results

UHPLC-Q-TOF-MS analysis of the aqueous polyherbal formulation (APE) revealed 60 bioactive compounds, of which 39 compounds exhibited antidiabetic activity. The APE has 6.465 mg/Q.E. g D.W. and 10.089 mg/GAE g D.W. of total flavonoids and phenolic content, respectively. APE scavenges oxygen free radicals, with IC_{50} values of 24.15 µg using DPPH, 25.485 µg using ABTS, and 39.95 µg using FRAP methods. A molecular docking study demonstrated the strong interaction of verbacoside B with the target proteins insulin-like growth factor-I (IGF-I) and GLUT4. This interaction suggests that the compound enhances insulin secretion and inhibits carbohydrate-digesting enzymes, subsequently increasing glucose uptake into cells and improving insulin sensitivity and overall glucose metabolism, thereby providing a multifaceted approach to managing diabetes. *In vitro* carbohydrate digestive enzyme inhibition assays revealed that APE significantly inhibited α-amylase and α-glucosidase at a minimal concentration. The calculated IC_{50} values were 54.26 ± 0.14 and 26.47 ± 0.12 µg/ml, respectively. Glucose uptake assays in MIN6 cells demonstrated that APE enhanced insulin secretion and glucose uptake in a dose-dependent manner, further supporting its antidiabetic efficacy. *In vivo,* acute and subacute oral toxicity studies revealed nontoxic nature of APE. At a dose of 500 mg/kg body weight, APE significantly decreased blood sugar and HbA1c levels and had no side effects on liver or kidney function, as measured by blood serum parameters. A histopathological study (H&E) revealed structural alterations in the pancreatic islets of the untreated diabetic control; however, a significant restoration near that of the normal control was observed in the APE-treated group. An immunohistochemical study revealed a 47% restoration of pancreatic β-cells following APE treatment in diabetic animal models, demonstrating the ability of APE to restore pancreatic function.

Gas chromatography–mass spectrometry (GC–MS) and Fourier transform infrared (FT–IR) spectroscopic analysis revealed the presence of several bioactive compounds in

the ayurvedic polyherbal formulation (APF). The quantitative analysis revealed a total phenolic content of 8.957 mg GAE/g DW and a total flavonoid content of 5.786 mg QE/g DW. The antioxidant efficacy of APF was assessed via DPPH, ABTS, and FRAP. The calculated IC_{50} values were 27.07, 41.31, and 25.92 µg/ml, respectively. *In vitro*, a carbohydrate digestive enzyme inhibition assay revealed that APF inhibits the α-amylase and α-glucosidase enzymes, with calculated IC_{50} values of 42.92 ± 0.24 and 30.60 ± 0.17 µg/ml, respectively. An oral toxicity study revealed 250 mg/kg body weight as the effective dose of APF, which does not affect other vital organs. The treated diabetic animals had significantly decreased blood glucose and HbA1c levels, suggesting that APF is capable of controlling postprandial blood glucose levels. A significant decrease in the size of islets was observed in the untreated diabetic control group (60–80 µm) compared with the normal control group (130–200 µm). In contrast, the APF-treated group presented significantly larger islet sizes (110–185 µm). Immunostaining with an anti-insulin antibody revealed 49.07% regeneration of pancreatic β-cells after APF treatment. The expression of the myosin Va molecular motor protein was significantly lower in the untreated diabetic control group (11.265 ± 2.15) than in the normal control group (70.285 ± 2.38). However, APF treatment restored its expression to 57.082 ± 1.28. The restoration of the myosin Va protein indicates its role in facilitating the movement of insulin granules towards the final stage of exocytosis, thereby normalizing hyperglycemia. Notably, the expression of myosin Va in the exocrine pancreas remained consistent across both the diabetic and treated groups (30.81 ± 1.44).

Fourier transform infrared (FT-IR) spectroscopy and gas chromatography–mass spectrometry (GC–MS) analysis of *Tinospora cordifolia* revealed the presence of diverse functional groups and a number of bioactive compounds with numerous biological activities, including antidiabetic activity. Quantitative analysis revealed that the extract contained 27.85 mg GAE/g DW of total phenolic content and 4.138 mg QE/g DW of total flavonoid content. The antioxidant activity of the extract was calculated in terms of DPPH free radical scavenging activity, with a calculated IC_{50} value of 5.40 µg/ml. *T. cordifolia* extract inhibited carbohydrate digestive enzymes (α-amylase and β-glucosidase) at a minimal concentration, which was comparable with standard ascorbic acid. The calculated IC_{50} values were 49.32 µg/ml and 44.36 µg/ml for α-amylase and β-glucosidase, respectively. A lipid accumulation assay using a 3T3-L1 cell line revealed that *T. cordifolia* extract inhibited lipid accumulation in a dose-dependent manner, and the calculated IC_{50} value was 48.68 µg/ml. Prior to the *in vivo* antidiabetic study of *T. cordifolia* extract, oral toxicity tests were conducted to determine the safe dose and

evaluate the toxic nature of the extract. The safe dose was calculated to be 250 mg/kg body weight, and the extract did not affect the other vital organs after administration. A physiochemical parameter study revealed that, compared with the untreated diabetic control group (123 ± 1.06 g), the extract-treated group (209.3 ± 1.24 g) presented a significant increase in body weight, confirming the positive effects of the extract on physiochemical parameters. A substantial reduction in blood glucose levels was observed in the extract-treated group (118 ± 5.35 mg/dl) compared with the diabetic control group (492 ± 0.98 mg/dl), illustrating its antidiabetic efficacy. The elevated kidney and liver functions observed during diabetes were restored to normal with the extract treatment, suggesting its broad spectrum of beneficial effects on various metabolic and organ-specific parameters under diabetic conditions. Histopathological and immunohistochemical studies revealed significant restoration of both synaptic integrity and insulin production in the pancreatic islets. In the extract-treated group, 34% of the β-cells regenerated.

Quantitative analysis of the *Mangifera indica* extract revealed total phenolic and flavonoid contents of 17.02 mg GAE/g and 2.35 mg QE/g D.W., respectively. The DPPH free radical scavenging activity assay revealed the highest scavenging activity of 97.09%, with a calculated IC_{50} value of 50.54 µg/ml. The *in vitro* carbohydrate digestive enzyme (β-glucosidase) inhibition assay revealed better inhibition activity with the extract treatment. The IC_{50} value was calculated to be 76.77 µg/ml, which was comparable with the standard. The oral toxicity study suggested that the safest dose for treatment was 200 mg/kg body weight, which is nontoxic to other vital organs. A positive effect of extract treatment was observed in managing elevated parameters such as body weight, blood glucose levels, and HbA1c levels. The biochemical serum marker levels were also well controlled with the extract treatment, suggesting its beneficial characteristic features of various metabolic and organ-specific parameters under diabetic conditions. Histopathological analysis revealed significant restoration of the structural architecture of the pancreatic islets in the extract-treated animals compared with those in the diabetic control animals. This result signifies the regenerative potential of the lost pancreatic β-cells.

Discussion & Conclusion

This study highlights the potential of polyherbal formulations (APE and APF) along with hydroalcoholic extracts of *T. cordifolia* and *M. indica* as promising adjunct therapies for diabetes management. Our *in silico*, *in vitro*, and *in vivo* analyses revealed that these natural products exert substantial antidiabetic effects through diverse mechanisms of

action. Secondary metabolites, including phenols and alkaloids, play a key role in inhibiting carbohydrate-digestive enzymes and improving lipid metabolism, further enhancing the therapeutic potential of these extracts.

A remarkable finding was the significant regeneration of β-cells and restoration of myosin Va expression in the treated groups compared with those in the controls. This regeneration within the pancreatic islets and recovery of β-cell function, along with the expression of myosin Va, a marker of insulin secretory potential, suggest an enhanced exocytotic capability within β-cells. These results underscore the ability of these herbal formulations to restore pancreatic architecture and functionality. Collectively, our findings support the use of these natural products as valuable therapeutic candidates for diabetes. Their multifaceted benefits complement conventional treatments, offering a powerful, natural option in diabetes management.