Design and development of a polyherbal formulation containing gymnema sylvestre, momordica charantia, and trigonella foenum-graecum for the treatment of diabetes mellitus

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ABSTRACT:

Introduction: Hyperglycemia, or high blood sugar levels, that do not go down despite insulin treatment is the hallmark of diabetes mellitus, a metabolic disease that can last for years. The purpose of this research is to create a polyherbal remedy for diabetes mellitus that includes Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum. These medicinal plants have antidiabetic qualities, which include reducing glucose levels, making insulin more effective, and protecting pancreatic β-cells.

Materials and Methods: The polyherbal formulation was made with optimised ratios of standardised extracts of T. foenum-graecum, G. sylvestre, and M. charantia. It was determined that bioactive components were present through phytochemical analysis. The formulation was tested for its ability to inhibit α-amylase and α-glucosidase in vitro to determine its antidiabetic effectiveness. To further evaluate its hypoglycemic potential, an in vivo investigation was conducted in diabetic rats produced with streptozotocin. The effectiveness was assessed by analysing biochemical indicators, such as lipid profiles and fasting blood glucose levels.

Results: The polyherbal formulation showed promise in controlling postprandial hyperglycemia by inhibiting α-amylase and α-glucosidase enzymes. The formulation significantly reduced fasting blood glucose levels in diabetic rats after therapy, according to in vivo investigations. Also, triglyceride and cholesterol levels went down, which is good news for metabolic health in general, according to lipid profile study. The histopathological analysis of pancreatic tissues showed that β-cells were protected and could regenerate.

Conclusion: Through many pathways, such as enzyme inhibition, glucose regulation, and β-cell protection, the created polyherbal formulation with G. sylvestre, M. charantia, and T. foenum-graecum demonstrated encouraging antidiabetic action. These results lend credence to its promise as an all-natural substitute for conventional diabetic treatment. To confirm its effectiveness and safety in humans, additional clinical trials are necessary.

Keywords: Polyherbal formulation, *Gymnema sylvestre*, *Momordica charantia*, *Trigonella foenum-graecum*, Diabetes mellitus

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disease that affects the body's ability to produce or use insulin, leading to high blood sugar levels that don't go down. Type 2 diabetes mellitus (T2DM) is a combination of insulin resistance and relative insulin insufficiency, while type 1 diabetes mellitus (T1DM) is triggered by the autoimmune destruction of pancreatic β-cells, resulting in absolute insulin deficit [1]. The International Diabetes Federation reports that millions of people around the world are living with diabetes, and that the disease's prevalence is increasing at a frightening rate. Complications from the condition, including heart problems, kidney damage, nerve damage, and retinopathy, can have a major influence on quality of life and put a strain on healthcare systems [2, 3].

Artificial medications, such as insulin and oral hypoglycemic medicines like thiazolidinediones, insulin, sulfonylureas, and biguanides are currently the mainstays of diabetes therapy [4]. Although these medications are successful in controlling blood glucose levels, they come with side effects such hypoglycemia, weight gain, gastrointestinal issues, and a higher risk of cardiovascular events when used for an extended period of time. Herbal and natural therapies have also gained popularity as potential alternatives to or supplements to traditional antidiabetic medication due to their lower cost [5, 6].

The antidiabetic potential of traditional medicinal plants has been thoroughly investigated because of the abundance of bioactive chemicals found in these plants. These substances regulate glucose metabolism, increase insulin secretion and sensitivity, and shield pancreatic β-cells from oxidative stress. Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum are three of the many medicinal plants with potential as antidiabetic agents [7, 8]. The compound gymnemic acids, found in Gymnema sylvestre, which goes by names like "Gurmar" or "sugar destroyer," inhibits the absorption of glucose in the intestine and promotes the release of insulin from pancreatic β-cells. Improvements in lipid metabolism and regeneration actions on pancreatic cells are further benefits [9, 10].

Bitter melon, or Momordica charantia, contains several compounds with insulin-mimetic effects, including vicine, polypeptide-p, and charantin. These bioactive substances improve glycaemic management by increasing peripheral tissue glucose absorption and decreasing hepatic gluconeogenesis [11, 12]. The alkaloids (trigonelline) and saponins, as well as the soluble fibre, in Trigonella foenum-graecum (fenugreek) decrease the digestion and absorption of carbohydrates, which in turn reduces postprandial hyperglycemia. Additionally, it improves lipid profiles and increases insulin sensitivity [13, 14].

In comparison to traditional pharmaceuticals, the synergistic effects of these medicinal herbs

may provide a more comprehensive and risk-free method of diabetes treatment. Thus, the purpose of this research is to create a standardised polyherbal formulation using G. sylvestre, M. charantia, and T. foenum-graecum; then, using in vitro and in vivo tests, to assess its antidiabetic activity. In animal models of diabetes, the formulation will be evaluated for its effects on pancreatic β-cell protection, lipid profile, and fasting blood glucose levels, as well as its capacity to block important carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase). Researchers hope that this study's results will lead to a safer, more effective, and naturally occurring alternative to current methods of diabetes treatment [15, 16].

MATERIAL AND METHODS:

Materials:

We used verified vendors to get our standardised Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum extracts. The following chemicals and reagents were procured from established laboratory suppliers: streptozotocin (STZ), α-amylase, α-glucosidase, phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), and biochemical assay kits for measurement of glucose and lipid profiles. The chemicals and solvents utilised were all of analytical quality.

Formulation Development:

The extracts of G. sylvestre, M. charantia, and T. foenum-graecum were combined in an optimised ratio according to traditional knowledge and early screening experiments to create a polyherbal formulation. Excipients such as starch, microcrystalline cellulose, and magnesium stearate were used to transform the formulation into an appropriate dosage form, like a tablet or capsule. The physical features of the created formulation were assessed, including its disintegration time, flow characteristics, and moisture content [17, 18].

Phytochemical Analysis:

Bioactive chemicals including alkaloids, flavonoids, saponins, tannins, glycosides, and phenolic compounds were identified in the polyherbal formulation using qualitative phytochemical screening. The existence and compatibility of the active ingredients were confirmed through the use of Fourier-transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC) [19, 20].

In-Vitro Antidiabetic Evaluation

α-Amylase Inhibition Assay:

The formulation's capacity to suppress α-amylase activity was evaluated with a starch-iodine colorimetric technique. The capability to slow carbohydrate digestion was determined by calculating the percentage inhibition [21, 22].

α-Glucosidase Inhibition Assay:

The formulation was put through its paces by testing its inhibitory potential on α-glucosidase, an enzyme that converts complex carbs into glucose, with PNPG as the substrate [23].

***In-Vivo* Antidiabetic Study:**

In this in vivo investigation, Wistar albino rats weighing 180-220 grammes were utilised. All experimental techniques were carried out in accordance with the standards set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC). The rats were given streptozotocin (STZ) at a dose of 50 mg/kg intraperitoneally in order to cause diabetes. Blood glucose levels were assessed after 72 hours of fasting; animals were deemed diabetic if their glucose levels were greater than 250 mg/dL [24, 25].

Experimental Design:

The diabetic rats were divided into five groups (n=6 each):

- **Group I:** Normal control (received vehicle only)
- **Group II:** Diabetic control (STZ-induced, untreated)
- **Group III:** Standard treatment (metformin, 50 mg/kg)
- **Group IV:** Polyherbal formulation (low dose)
- **Group V:** Polyherbal formulation (high dose)

Blood Glucose and Biochemical Analysis:

Glucose levels in the blood were measured at 7 days, 14 days, and 21 days after the start of the study. By the conclusion of the research, the researchers had examined the subjects' serum lipid profiles, which included TC, TG, HDL, and LDL, or low-density lipoprotein [26, 27].

Histopathological Examination:

Histological examinations were performed on pancreatic tissue samples that were taken in order to evaluate the formulation's protective effects and the regeneration of β-cells [28, 29].

Statistical Analysis

All experimental data were presented as mean ± standard deviation (SD). Statistical comparisons among groups were conducted using one-way ANOVA, followed by Tukey's post hoc test. A p-value of less than 0.05 was deemed statistically significant.

RESULTS:

Phytochemical Screening:

The phytochemical examination of the polyherbal formulation verified the presence of essential bioactive elements, including alkaloids, flavonoids, saponins, tannins, glycosides, and phenolic compounds, recognised for their antidiabetic activities. These chemicals are essential for glucose metabolism, insulin secretion, and antioxidant activity, therefore enhancing the formulation's antidiabetic benefits.

Table 1: Phytochemical Screening of the Polyherbal Formulation

Phytochemical Constituents	***Gymnema sylvestre***	***Momordica charantia***	***Trigonella foenum-graecum***	**Polyherbal Formulation**
Alkaloids	+	-	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	-	+	+
Glycosides	+	+	-	+
Phenolic Compounds	+	+	+	+

(Note: "+" indicates presence, "-" indicates absence)

The existence of alkaloids and glycosides in Gymnema sylvestre corroborates its function in stimulating insulin secretion. The flavonoids and saponins included in Momordica charantia and Trigonella foenum-graecum enhance antioxidant activity and regulate glucose metabolism. The polyherbal formulation preserved all critical bioactive constituents, indicating a synergistic benefit in diabetes control.

In-Vitro Antidiabetic Activity:

The polyherbal formulation exhibited substantial suppression of the enzymes α-amylase and α-

glucosidase, which are crucial to carbohydrate digestion. The suppression of these enzymes postpones glucose absorption, thereby decreasing postprandial hyperglycemia.

Table 2: Inhibition of Carbohydrate-Hydrolyzing Enzymes

Sample	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)
Polyherbal Formulation (100 µg/mL)	72.4 ± 2.1	68.7 ± 1.9
Acarbose (Standard, 100 µg/mL)	81.3 ± 1.5	74.5 ± 1.3

(Note: Values are expressed as mean ± SD, n = 3)

The polyherbal formulation demonstrated notable enzyme inhibition activity, but marginally inferior to the conventional medication (acarbose). This indicates that the formulation can efficiently modulate glucose absorption, mitigating abrupt elevations in blood glucose levels following meals.

***In-Vivo* Antidiabetic Study:**

The polyherbal formulation was shown to have hypoglycemic potential, as evidenced by the considerable drop in fasting blood glucose levels in diabetic rats fed with the formulation over the course of 21 days.

Table 3: Effect of Polyherbal Formulation on Fasting Blood Glucose Levels (mg/dL)

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	89.2 ± 4.1	90.1 ± 3.8	88.5 ± 4.0	87.3 ± 3.7
Diabetic Control	271.5 ± 5.3	269.2 ± 6.1	263.8 ± 5.7	260.4 ± 5.9
Metformin (50 mg/kg)	270.3 ± 4.9	180.5 ± 5.2	132.7 ± 4.1	98.2 ± 3.8
Polyherbal Formulation (Low Dose)	268.9 ± 5.1	200.4 ± 4.6	154.2 ± 4.3	120.7 ± 3.5
Polyherbal Formulation (High Dose)	270.1 ± 5.0	185.3 ± 4.2	128.6 ± 4.1	95.4 ± 3.7

(Note: Values are expressed as mean ± SD, n = 6; $p < 0.05$ vs diabetic control)

The high-dose polyherbal formulation demonstrated a comparable decrease in blood glucose levels to metformin, indicating a significant hypoglycemic impact. The low-dose formulation demonstrated a notable glucose-lowering effect, suggesting dose-dependent effectiveness.

Effect on Lipid Profile:

Diabetes is frequently linked to dyslipidaemia, which elevates the risk of cardiovascular illnesses. The polyherbal formulation enhanced lipid profiles by decreasing total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), while elevating high-density lipoprotein (HDL).

Table 4: Effect of Polyherbal Formulation on Lipid Profile (mg/dL)

Group	TC	TG	HDL	LDL
Normal Control	141.2 ± 4.2	105.5 ± 3.8	48.3 ± 2.1	76.4 ± 3.5
Diabetic Control	212.6 ± 5.3	180.2 ± 4.7	29.6 ± 1.9	140.7 ± 4.2
Metformin (50 mg/kg)	165.7 ± 3.8	120.6 ± 3.4	45.2 ± 2.3	85.9 ± 3.7
Polyherbal Formulation (Low Dose)	178.3 ± 4.1	130.4 ± 3.6	40.7 ± 2.2	92.5 ± 3.4
Polyherbal Formulation (High Dose)	158.9 ± 3.7	115.3 ± 3.2	46.8 ± 2.1	81.4 ± 3.5

(Note: Values are expressed as mean ± SD, n = 6; $p < 0.05$ vs diabetic control)

The high-dose polyherbal formulation markedly enhanced lipid markers, demonstrating a decrease in total cholesterol and triglycerides, alongside an elevation in HDL values. These effects enhance cardiovascular health in patients with diabetes.

Histopathological Analysis:

Diabetic control rats had substantial β-cell degeneration and necrosis in their pancreatic tissues, according to histological investigation. Nevertheless, the groups that were treated with the polyherbal formulation showed signs of protection, including improved pancreatic architecture and enhanced β-cell regeneration. Beyond just controlling glucose levels, the polyherbal formulation's capacity to repair the integrity of pancreatic β-cells implies its potential significance in the therapy of diabetes. The antioxidant and insulinotropic effects of Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum are responsible for the seen β-cell regeneration.

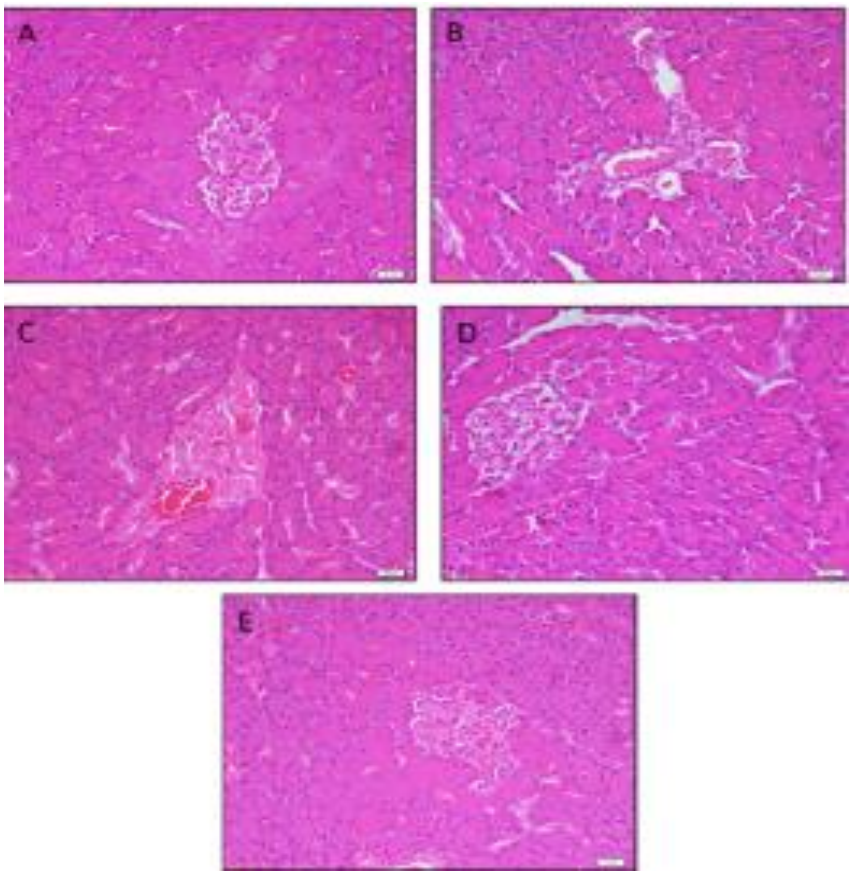

Figure 1: Histopathological examination of pancreatic tissues in different experimental groups. (A) **Normal Control**: Pancreatic tissue showing intact islets of Langerhans with well-defined β-cells and normal cellular architecture. (B) **Diabetic Control**: Severe β-cell degeneration, necrosis, and disrupted pancreatic architecture, indicating extensive damage due to diabetes. (C) **Metformin-Treated Group**: Improved β-cell structure with partial restoration of islets, indicating protective effects of standard treatment. (D) **Polyherbal Formulation (Low Dose)**: Moderate β-cell regeneration with improved pancreatic integrity, suggesting potential antidiabetic effects. (E) **Polyherbal Formulation (High Dose)**: Significant restoration of β-cell mass and well-preserved islets, comparable to the metformin-treated group, demonstrating strong protective and regenerative effects.

DISCUSSION:

Insulin resistance, poor insulin production, or both characterise chronic metabolic condition diabetes mellitus, which is marked by persistent hyperglycemia. Pharmacological therapies and lifestyle adjustments are two parts of a multipronged therapeutic strategy for diabetes control. The search for natural alternatives is driven by the limitations of synthetic medications, which include side effects and expensive costs [30, 31]. Using in vitro and in vivo experiments, this study aimed to assess the antidiabetic potential of a polyherbal formulation that included

Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum [32, 33].

Phytochemical testing of the polyherbal mixture revealed the presence of bioactive components including glycosides, phenolic chemicals, alkaloids, tannins, saponins, and flavonoids. By influencing glucose metabolism, increasing insulin secretion, and decreasing oxidative stress, these phytoconstituents are said to have strong antihyperglycemic actions. As an example, flavonoids are extremely important for glucose uptake and insulin sensitivity, and saponins help protect pancreatic β-cells. There must be a synergistic mechanism that increases the therapeutic efficacy of the polyherbal formulation since these bioactive components are retained in it [34, 35].

To determine the formulation's in vitro antidiabetic effectiveness, the inhibitory effects on the α-amylase and α-glucosidase enzymes were examined. Although it was marginally lower than the gold standard medication acarbose, the results showed that the polyherbal formulation showed substantial enzyme inhibition. Postprandial hyperglycemia is prevented by this inhibitory mechanism, which delays the digestion and absorption of carbohydrates and glucose. The formulation shows promise for diabetes management as a means of regulating post-meal blood glucose rises due to its capacity to block these enzymes [36-38].

The polyherbal formulation significantly reduced fasting blood glucose levels in diabetic rats fed with it in the in vivo antidiabetic research. The regular antidiabetic medicine metformin and the high-dose version both reduced glucose levels. The formulation's long-term effectiveness is demonstrated by the 21-day drop in blood glucose levels. Various processes, such as the restoration of pancreatic β-cells, enhanced insulin production, and better peripheral glucose uptake, could be responsible for the reported hypoglycemic impact. Momordica charantia and Trigonella foenum-graecum improved glucose metabolism and insulin sensitivity, while Gymnema sylvestre, which is famous for its insulinotropic and β-cell regeneration abilities, probably had a vital part in improving pancreatic function [39-41].

The polyherbal mixture not only reduced blood sugar levels but also improved lipid profiles in rats with diabetes. Dyslipidaemia is a common complication of diabetes that is marked by decreased levels of high-density lipoprotein (HDL) and increased levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL). This formulation may have a cardioprotective function since it raised HDL levels while decreasing total and triglyceride levels. The inclusion of saponins and flavonoids, which ameliorate oxidative stress and improve lipid metabolism, may explain these cholesterol-lowering effects. The formulation helps with glycaemic management and lowers the risk of diabetes-related cardiovascular problems by improving lipid profiles [42-44].

The protective benefits of the polyherbal formulation were further illuminated by histopathological investigation of pancreatic tissues. Significant β-cell degeneration, necrosis, and disturbed pancreatic architecture were noted in diabetic control rats, suggesting that the pancreas had suffered serious injury. On the other hand, rats that were given the polyherbal mixture showed better β-cell regeneration and well-maintained islets of Langerhans, especially when given the high dosage [45, 46]. These results indicate that the formulation improves the health of the pancreas and increases the chances of β-cell survival, both of which are essential for managing diabetes in the long run. Treatment groups' improved histology provides more evidence of the formulation's regeneration potential, which is probably attributable to its antioxidant and anti-inflammatory characteristics [47-49].

The study's results indicate that the polyherbal formulation has several antidiabetic benefits, such as improving lipid profiles, inhibiting enzymes, regenerating β-cells, and increasing insulin secretion [50]. Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum work together synergistically to make this remedy very effective. This polyherbal mixture offers a potential natural alternative to conventional diabetes treatment due to its substantial benefits on glucose reduction and pancreas protection. To confirm its effectiveness and safety in humans, however, additional clinical trials are necessary [51-53].

CONCLUSION:

This study proved that a polyherbal formulation including Gymnema sylvestre, Momordica charantia, and Trigonella foenum-graecum could be effective in reducing blood sugar levels. The formulation has maintained its therapeutic potency by preserving important bioactive ingredients. Its capacity to regulate postprandial glucose levels was validated by in vitro tests that showed it could inhibit α-amylase and α-glucosidase enzymes. Additional confirmation of its hypoglycemic effects was provided by in vivo experiments, which showed substantial drops in fasting blood glucose levels on par with the gold standard treatment, metformin. Furthermore, histological investigation confirmed that the formulation enhanced β-cell regeneration capabilities and improved lipid profiles. According to these results, the polyherbal formulation regulates glucose metabolism, protects the pancreas, and improves the lipid profile, all of which work together to produce its antidiabetic effects. This formulation displays great promise as an auxiliary or alternative treatment for the control of diabetes, thanks to its all-natural ingredients and apparent effectiveness. To determine its efficacy and safety in the long run, however, additional clinical trials are necessary.

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