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Research Article

Pharmacological Evaluation of Antidiabetic Activity of Novel Herbal Combination in Experimental Model

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Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Despite the availability of several synthetic antidiabetic agents, their long-term use is often associated with adverse effects, necessitating the exploration of safer and more effective alternatives. Herbal medicines have gained considerable attention due to their therapeutic efficacy, affordability, and minimal side effects. The present study aims to evaluate the antidiabetic activity of a novel herbal combination in an experimental animal model. Diabetes was induced in experimental animals using a standard diabetogenic agent, and the animals were divided into normal control, diabetic control, standard drug-treated, and herbal combination-treated groups. The herbal formulation was administered orally for a specified period, and various biochemical parameters such as fasting blood glucose, body weight, lipid profile, and serum insulin levels were assessed. Histopathological examination of pancreatic tissue was also performed to evaluate protective effects on β-cells. The results demonstrated a significant reduction in blood glucose levels and improvement in associated metabolic parameters in the herbal combination-treated group compared with the diabetic control group. Histological findings further supported the antidiabetic potential of the formulation. The study concludes that the novel herbal combination possesses promising antidiabetic activity and may serve as a potential therapeutic alternative for the management of diabetes mellitus.

Keywords: Diabetes mellitus, Herbal combination, Antidiabetic activity, Experimental model, Hyperglycemia, Phytotherapy.

INTRODUCTION

Diabetes mellitus is one of the most prevalent endocrine disorders worldwide and is characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. According to the International Diabetes Federation, the incidence of diabetes continues to rise globally, posing a significant public health challenge. Long-term uncontrolled diabetes can lead to severe complications such as cardiovascular diseases, nephropathy, neuropathy, retinopathy, and impaired wound healing. [1] Current antidiabetic therapies, including insulin and oral hypoglycemic agents, effectively control blood glucose levels but may cause adverse effects such as hypoglycemia, weight gain, gastrointestinal disturbances, and hepatic toxicity. Therefore, there is increasing interest in identifying natural products with antidiabetic potential that can provide effective glycemic control with fewer side effects. [2] Medicinal plants have been used traditionally for the treatment of diabetes for centuries. Various phytoconstituents, including flavonoids, alkaloids, glycosides, tannins, terpenoids, and phenolic compounds, have demonstrated significant antihyperglycemic activity through multiple mechanisms such as stimulation of insulin secretion, enhancement of glucose uptake, inhibition of carbohydrate digestion, and antioxidant activity. [3] A novel herbal combination consisting of selected medicinal plants with complementary mechanisms of action may provide synergistic therapeutic benefits. The present investigation was designed to evaluate the pharmacological antidiabetic activity of a novel

herbal combination using an experimentally induced diabetic animal model and compare its efficacy with a standard antidiabetic drug. [5]

Table 1: Comparative Pharmacognostic and Pharmacological Profile of Selected Plants Used in the Novel Herbal Antidiabetic Formulation.

Parameter	Guduchi (Tinospora cordifolia)	Flax (Linum usitatissimum)	Garlic (Allium sativum)
Family	Menispermaceae	Linaceae	Amaryllidaceae
Plant Type	Climbing shrub	Annual herb	Bulbous herb
Main Active Constituents	Alkaloids, Diterpenoids, Flavonoids	Omega-3 Fatty Acids, Lignans	Allicin, Ajoene, Organosulfur Compounds
Antidiabetic Activity	Enhances insulin secretion and β-cell protection	Improves insulin sensitivity and glucose metabolism	Reduces blood glucose and oxidative stress
Antioxidant Activity	Strong	Strong	Strong
Additional Benefits	Immunomodulatory, Hepatoprotective	Cardioprotective, Hypolipidemic	Antimicrobial, Cardioprotective
Part Used	Stem and leaves	Seeds	Bulb
Method of Propagation	Stem cuttings	Seeds	Cloves

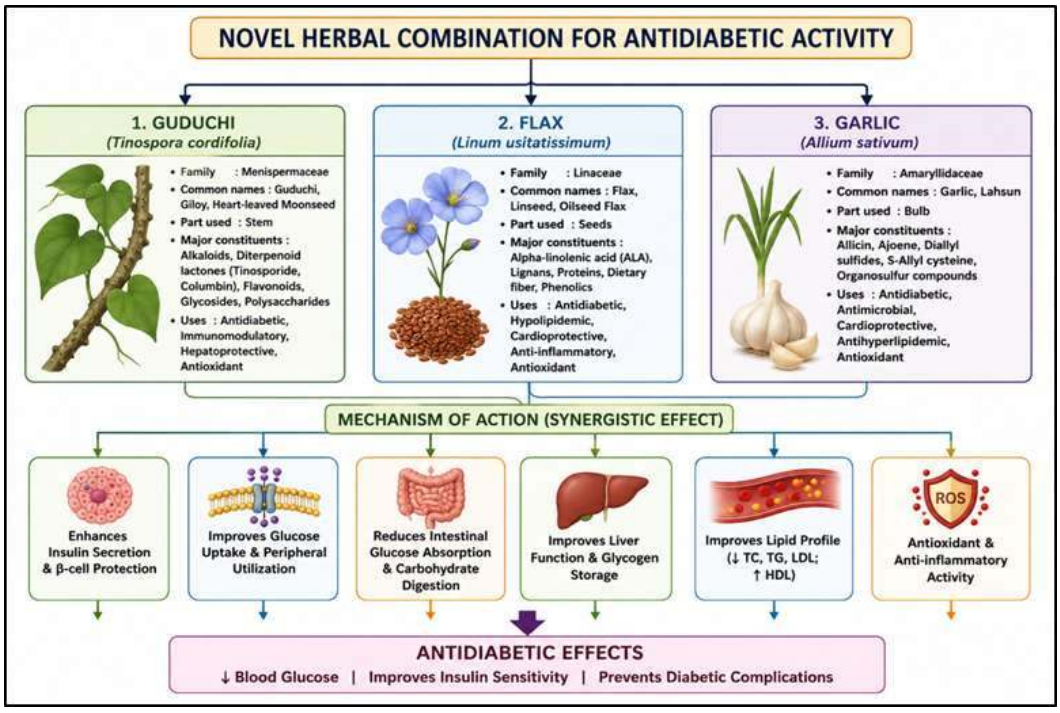

Figure 1. Schematic Representation of the Antidiabetic Activity of the Novel Herbal Combination.

MATERIALS

The stem of Tinospora cordifolia (Guduchi), seeds of Linum usitatissimum (Flax), and bulbs of Allium sativum (Garlic) were used for the preparation of the novel herbal formulation. Streptozotocin (STZ) was used for the induction of diabetes, while Metformin hydrochloride was used as the standard antidiabetic drug. Ethanol (70%), distilled water, citrate buffer (pH 4.5), and commercially available biochemical diagnostic kits were employed for extraction and biochemical estimations. Healthy Wistar albino rats (150–250 g) of either sex were used as experimental animals. The study also utilized standard laboratory

equipment including a Soxhlet extraction apparatus, rotary vacuum evaporator, electronic balance, glucometer, centrifuge, UV–Visible spectrophotometer, and microscope for experimental procedures and analysis.

METHODOLOGY

Collection and Authentication of Plant Materials

The flax seeds (*Linum usitatissimum*), fresh garlic bulbs (*Allium sativum*), and stems of Guduchi (*Tinospora cordifolia*) were procured from the local market of Chikhali, District Buldana, Maharashtra, India. The plant materials were authenticated by Dr. Pramod R. Padole, Professor of Botany, Shivaji Science and Arts College, Chikhali, Buldana. Voucher specimens (ACP/HF-489, ACP/HF-490, and ACP/HF-491) were deposited in the Herbarium Department for future reference.The authenticated plant materials were cleaned, shade-dried at room temperature under controlled humidity conditions until free from moisture, and then pulverized using a mechanical grinder. The powdered materials were passed through sieve No. 40 and stored in airtight containers for further pharmacognostic, phytochemical, and pharmacological investigations.

Morphological and Microscopic Evaluation

The crude drugs were evaluated for organoleptic characteristics including colour, odour, taste, shape, and size. Microscopic examination of flax seeds, Guduchi stem, and garlic bulb was carried out using suitable staining reagents and observed under a compound microscope to confirm their diagnostic characteristics. [6]

Powder Microscopy

The powdered plant materials were passed through sieve No. 60. A small quantity of powder was mounted on a glass slide with suitable reagents such as water, glycerin, iodine solution, or phloroglucinol-HCl. The slides were observed under low (10×) and high (40×) magnifications for identification of characteristic cellular structures. [7]

Fluorescence Analysis

One gram of powdered drug was treated separately with different reagents including distilled water, 1N sodium hydroxide, 1N hydrochloric acid, nitric acid, sulfuric acid, chloroform, ethanol, and ammonia solution. The resulting colour changes were observed under daylight and ultraviolet light (254 nm and 366 nm) for authentication and detection of adulteration. [8]

Extraction of Plant Materials

The powdered flax seeds were defatted with petroleum ether and extracted with 80% ethanol using a Soxhlet apparatus. The extract was concentrated using a rotary evaporator. The powdered stems of *Tinospora cordifolia* were defatted with petroleum ether and extracted using hydroalcoholic solvent (ethanol, 80:20) by ultrasound-assisted extraction. The extract was filtered, concentrated under reduced pressure, and stored at 4°C.Fresh garlic cloves were peeled, crushed, and allowed to stand for 10–15 minutes to facilitate allicin formation. Extraction was carried out using ethanol (60:40) under ultrasound-assisted conditions. The extract was concentrated under reduced pressure below 35°C and stored at 4°C until use. [9]

Micromeritic Evaluation

The powdered extracts were evaluated for mean particle size, angle of repose, bulk density, tapped density, compressibility index, and Hausner ratio using standard procedures to determine flow properties and packing characteristics. [10]

Heavy Metal Analysis

Quantitative estimation of heavy metals including arsenic, cadmium, mercury, and lead was carried out according to WHO guidelines to ensure the safety and quality of **Table [11]**

Experimental Animals

Healthy male Wistar albino rats (130–180 g, 2–2.5 months old) were used for the study. Animals were housed in polypropylene cages under standard laboratory conditions (25 ± 2°C, 12 h light/dark cycle) with free access to standard pellet diet and water. The animals were acclimatized for seven days before the

experiment. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines. [12]

Experimental Design

Thirty rats were divided into five groups (n = 6):

Group	Treatment
Group I	Normal Control (Saline)
Group II	Diabetic Control (STZ 40 mg/kg, i.p.)
Group III	Standard (Metformin 50 mg/kg, p.o.)
Group IV	Polyherbal Formulation Low Dose (50 mg/kg, p.o.)
Group V	Polyherbal Formulation High Dose (200 mg/kg, p.o.)

Table 3. Formulation of polyherbal formulation

Sr. No.	Ingredient	Tablet Formulation		Use
		F1 (Low dose)	F2 (High dose)	
1.	*Allium sativum extract*	45 mg	75 mg	Antidiabetic
2.	*Tinospora cardifolia extract*	30 mg	135 mg	Antidiabetic
3.	*Linum usitatissimum extract*	15 mg	60 mg	Antidiabetic
4.	Spray dried lactose	96 mg	09 mg	Diluent
5.	Sodium starch glycolate	4 mg	6 mg	Disintegrant
6.	Magnesium stearate	4 mg	6 mg	Lubricant

Induction of Diabetes

Diabetes was induced by intraperitoneal administration of Streptozotocin (STZ) (60 mg/kg) dissolved in citrate buffer (pH 4.5), following nicotinamide (110 mg/kg, i.p.). After one week, rats with fasting blood glucose levels above 200 mg/dL were considered diabetic and included in the study. [14]

Oral Glucose Tolerance Test (OGTT)

Following overnight fasting, animals received glucose (2 g/kg, p.o.) 30 min after administration of saline, standard drug, or test formulation. Blood glucose levels were measured at 0, 0.5, 1, 2, 3, and 4 h using a glucometer. [15]

Hypoglycemic Activity

Based on OGTT results, diabetic rats were treated orally with Metformin or the polyherbal formulation once daily for 28 days. Fasting blood glucose levels and body weight were recorded periodically throughout the study. [16]

Evaluation Parameters [17]

- Fasting Blood Glucose (FBG)
- Oral Glucose Tolerance Test (OGTT)
- Body Weight
- Lipid Profile
- Serum Insulin
- Liver and Kidney Function Tests
- Oxidative Stress Markers
- Histopathological Examination of Pancreas and Liver

Histopathological Study

At the end of the study, pancreas and liver tissues were collected and fixed in 10% formalin. The tissues were processed, embedded in paraffin wax, sectioned at 5 µm thickness, stained with Hematoxylin and Eosin (H&E), and examined under a light microscope for histopathological changes [18]

Statistical Analysis

Data were expressed as Mean ± SEM and analyzed using One-Way ANOVA followed by Dunnett's multiple comparison test. Values of $\mathbf{p < 0.05}$ were considered statistically significant. [18]

Figure 2. Experimental Design for Evaluation of Antidiabetic Activity of Novel Polyherbal Formulation in STZ-Induced Diabetic Rats.

RESULTS

Fluorescence Analysis

Fluorescence analysis of *Tinospora cordifolia*, *Allium sativum*, and *Linum usitatissimum* powders was carried out using various chemical reagents under daylight and UV light (366 nm). The observed fluorescence characteristics were found to be consistent with standard pharmacognostic parameters and showed no evidence of adulteration. The results confirmed the identity and purity of the selected crude drugs.

Table 4. Fluorescence Analysis of Crude Drugs

Crude Drug	Reagent	Daylight Colour	UV (366 nm) Fluorescence
Guduchi	Powder alone	Brownish-green	Greenish-yellow
	NaOH (Aqueous)	Yellowish	Bright yellow
	HCl	Brown	Dull red
Garlic	Powder alone	Cream	Bluish
	Ethanol Extract	Pale yellow	Green
	NaOH (Aqueous)	Yellow	Bright green
Flax Seeds	Powder alone	Brown	No fluorescence
	NaOH (Methanolic)	Light brown	Faint green
	Dilute H_2SO_4	Blackish	No fluorescence

Extraction Yield

Hydroalcoholic extracts of *Linum usitatissimum*, *Tinospora cordifolia*, and *Allium sativum* were prepared using suitable extraction techniques. The percentage yield of extracts was calculated based on the weight of dried extract obtained from 100 g of crude drug.

Table 5 Percentage Yield of Plant Extracts

Sr. No.	Plant Name	Weight of Extract (g)	Crude Drug Used (g)	% Yield
1	*Linum usitatissimum*	18.32 ± 3.18	100	18.32 ± 3.18
2	*Tinospora cordifolia*	23.54 ± 2.81	100	23.54 ± 2.81
3	*Allium sativum*	13.27 ± 1.37	100	13.27 ± 1.37

Values are expressed as Mean ± SD (n = 3).

Among the selected plants, *Tinospora cordifolia* exhibited the highest extractive yield

(23.54 ± 2.81%), followed by *Linum usitatissimum* (18.32 ± 3.18%), whereas *Allium sativum* showed the lowest yield (13.27 ± 1.37%). The extraction methods employed provided stable and concentrated extracts suitable for further phytochemical and pharmacological evaluation.

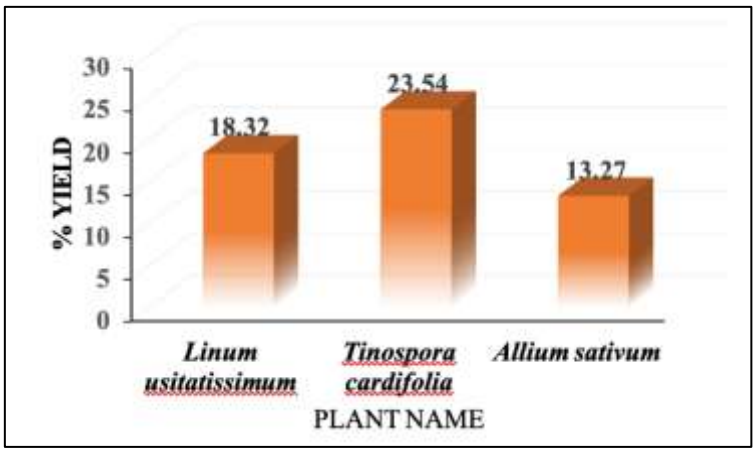

Figure 3: Percentage Yield of Hydroalcoholic Extracts of *Tinospora cordifolia*, *Linum usitatissimum*, and *Allium sativum*.

Physical Properties of Extracts

The prepared extracts of Linum usitatissimum, Tinospora cordifolia, and Allium sativum were evaluated for their physical characteristics, including colour, pH, and texture. The extracts exhibited characteristic colours ranging from reddish-brown to yellowish-green and showed a soft sticky texture. The pH of aqueous extracts ranged between 6.2 and 6.8, indicating their suitability for further pharmaceutical applications.

Table 6: Physical Properties of Plant Extracts

Plant	Part Used	Colour	pH	Texture
Linum usitatissimum	Seeds	Reddish-brown	6.8	Sticky soft
Tinospora cordifolia	Stem	Slightly green	6.5	Sticky soft
Allium sativum	Bulb	Yellowish	6.2	Sticky soft

Micromeritic Properties of Extracts

Micromeritic evaluation revealed that all extracts possessed acceptable flow and compressibility characteristics. The angle of repose values were below 30°, indicating good flow properties, while Hausner's ratio and compressibility index suggested fair to good powder flow behavior.

Table 7 Micromeritic Properties of Extracts

Parameter	*L. usitatissimum*	*T. cordifolia*	*A. sativum*
Particle Size (µm)	635	718	532
Angle of Repose (°)	28.32	26.64	25.42
Bulk Density (g/cc)	0.534	0.596	0.563
Tapped Density (g/cc)	0.625	0.702	0.741
Compressibility Index (%)	14.56	15.10	24.02
Hausner's Ratio	1.17	1.18	1.31

Values represent mean of triplicate determinations.

Physicochemical Evaluation

Physicochemical parameters such as loss on drying, ash values, and extractive values were determined to assess the quality and purity of the extracts. The results indicated acceptable moisture content and ash values, while higher water-soluble and alcohol-soluble extractive values suggested the presence of significant amounts of polar phytoconstituents.

Table 8: Physicochemical Evaluation of Plant Extracts (% w/w)

Parameter	*L. usitatissimum*	*T. cordifolia*	*A. sativum*
Loss on Drying	8.32	10.64	5.42
Total Ash	3.43	7.96	4.26
Acid Insoluble Ash	2.12	1.08	3.29
Water Soluble Ash	0.87	5.18	1.74
Water Soluble Extractive	14.11	27.84	31.85
Alcohol Soluble Extractive	7.61	3.10	4.39
Ether Soluble Extractive	3.37	0.91	22.50

The obtained values confirmed the quality, purity, and suitability of the extracts for further phytochemical and pharmacological investigations.

Quantitative Estimation of Heavy Metals

Heavy metal analysis was performed according to WHO guidelines to evaluate the safety of the selected plant materials. The concentrations of lead, mercury, arsenic, iron, and cadmium were found to be within permissible limits, indicating that the extracts were safe for pharmacological studies.

Table 9: Heavy Metal Content of Plant Extracts (mg/kg)

Metal	*L. usitatissimum*	*T. cordifolia*	*A. sativum*
Lead (Pb)	5.01	4.13	6.13
Mercury (Hg)	0.02	0.01	0.03
Arsenic (As)	0.013	0.005	0.017
Iron (Fe)	2.32	1.38	4.51
Cadmium (Cd)	0.16	0.18	0.21

The results demonstrated that all heavy metal concentrations were below the permissible limits prescribed by WHO, confirming the safety and quality of the plant materials used in the study.

Formulation and Evaluation of Novel Polyherbal Tablets

The polyherbal tablets were prepared using extracts of *Linum usitatissimum*, *Tinospora cordifolia*, and *Allium sativum*along with spray-dried lactose, sodium starch glycolate, magnesium stearate, talc, and aerosil. Due to the sticky nature of the extracts, no additional binder was required. The prepared formulations (F1 and F2) were evaluated for organoleptic and physical parameters. Both formulations complied with official pharmacopoeial limits for hardness, friability, thickness, and disintegration time.

Table 10 Evaluation of Polyherbal Tablet Formulations

Parameter	F1 (Low Dose)	F2 (High Dose)
Colour	Greenish-brown	Greenish-brown
Odour	Characteristic aromatic	Characteristic aromatic
Hardness (kg/cm²)	4.5 ± 0.5	4.0 ± 0.5
Friability (%)	0.51	0.73
Thickness (mm)	3.12 ± 0.08	3.27 ± 0.18
Disintegration Time	2 min 14 sec	2 min 46 sec

Antidiabetic Activity of Polyherbal Formulation

The antidiabetic activity of the polyherbal formulation was evaluated in streptozotocin (STZ)-induced diabetic Wistar rats following OECD-423 guidelines. Blood glucose levels, food intake, water intake, and body weight were monitored throughout the 28-day study period.

Table 11 Parameters Monitored During Study

Parameter	Observation Interval
Blood Glucose Level	Days 7, 14, 21, and 28
Food Intake	Daily
Water Intake	Daily
Body Weight	Weekly

Oral Glucose Tolerance Test and Hypoglycemic Activity

The polyherbal formulation demonstrated dose-dependent glucose-lowering activity in glucose-loaded and STZ-induced diabetic rats. Significant reductions in blood glucose levels were observed after continuous administration for 28 days. The effect was comparable to the standard antidiabetic drug, Glibenclamide (5 mg/kg).

Table 12 Effect of Polyherbal Formulation on Blood Glucose Levels (mg/dL)

Group	Initial	Day 7	Day 14	Day 21	Day 28
Normal Control	90.97 ± 1.47	94.97 ± 1.47	104.3 ± 4.1	102.0 ± 2.13	89.52 ± 2.16
Diabetic Control	104.22 ± 0.22	259.3 ± 3.51	303.2 ± 5.53	375.1 ± 1.30	405.3 ± 1.26
Glibenclamide (5 mg/kg)	104.12 ± 1.20	267.8 ± 3.15	150.1 ± 1.42	143.2 ± 0.54	123.5 ± 1.03
Formulation (50 mg/kg)	94.10 ± 5.57	260.84 ± 1.57	168.1 ± 1.22	145.0 ± 2.27	148.0 ± 4.74

The diabetic control group showed a continuous increase in blood glucose levels, whereas treatment with the polyherbal formulation significantly reduced glucose levels from Day 14 onwards. The formulation exhibited antidiabetic activity comparable to Glibenclamide. This effect may be attributed to the presence of phenolic and flavonoid compounds, which improve insulin sensitivity, reduce oxidative stress, and enhance glucose metabolism. The results suggest that the developed polyherbal formulation possesses promising antidiabetic potential.

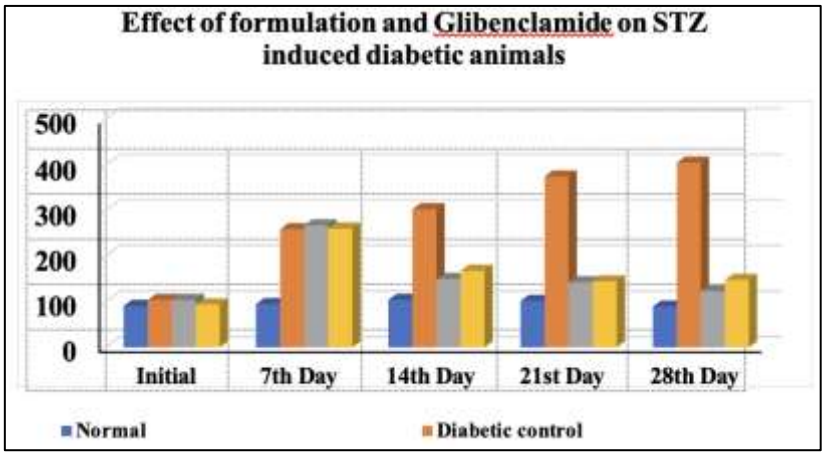

Figure 3 Effect of formulation and Glibenclamide on STZ induced diabetic animals

Table 13 Comparative Antidiabetic Activity of Glibenclamide and Formulation

Parameter	Glibenclamide Treated Group	Formulation Treated Group
7th Day Blood Glucose Level	267.8 ± 3.15	260.8 ± 1.57
14th Day Blood Glucose Level	150.1 ± 1.42	168.1 ± 1.22
21st Day Blood Glucose Level	143.2 ± 0.54	145 ± 2.27
28th Day Blood Glucose Level	123.5 ± 1.03	148 ± 4.74
Percentage Reduction (7th–14th Day)	43.99%	35.54%
Percentage Reduction (7th–21st Day)	46.53%	44.4%
Percentage Reduction (7th–28th Day)	53.88%	43.5%

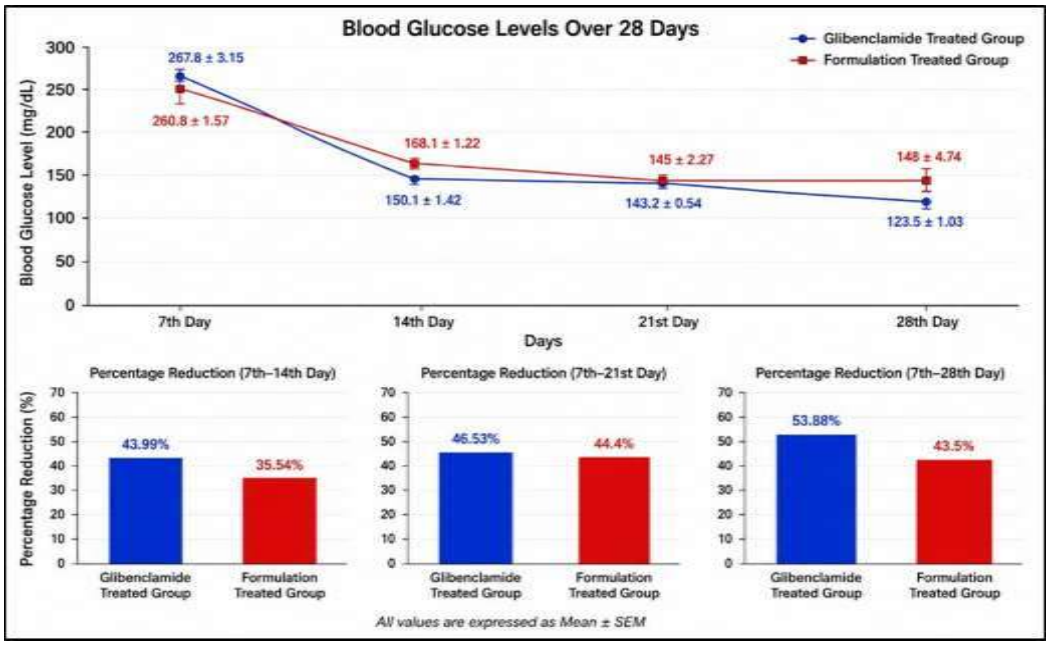

Figure 4 Comparative Antidiabetic Activity of Glibenclamide and Formulation

Blood Glucose Levels

The fasting and non-fasting blood glucose levels were significantly increased in the diabetic control group throughout the study. Treatment with Glibenclamide and the polyherbal formulation significantly reduced blood glucose levels compared to diabetic control animals. Although Glibenclamide produced a greater reduction, the test formulation showed considerable antihyperglycemic activity and effectively controlled both fasting and postprandial blood glucose levels. These findings indicate the potential antidiabetic efficacy of the developed polyherbal formulate

Table 14 Blood Glucose Levels

Group	Fasting Blood Glucose (Day 28, mg/dL)	Non-Fasting Blood Glucose (Day 28, mg/dL)	Observation
Group I – Normal Control	89.7 ± 1.12	114.6 ± 1.35	Normal glucose levels maintained
Group II – Diabetic Control	310.2 ± 4.64	378.8 ± 6.12	Significant increase in glucose levels
Group III – Diabetic + Glibenclamide	123.5 ± 1.03	160.8 ± 1.42	Maximum reduction in glucose levels
Group IV – Diabetic + Test Formulation	148.0 ± 4.74	176.2 ± 2.04	Significant antihyperglycemic activity

Values expressed as Mean ± SEM (n = 6).

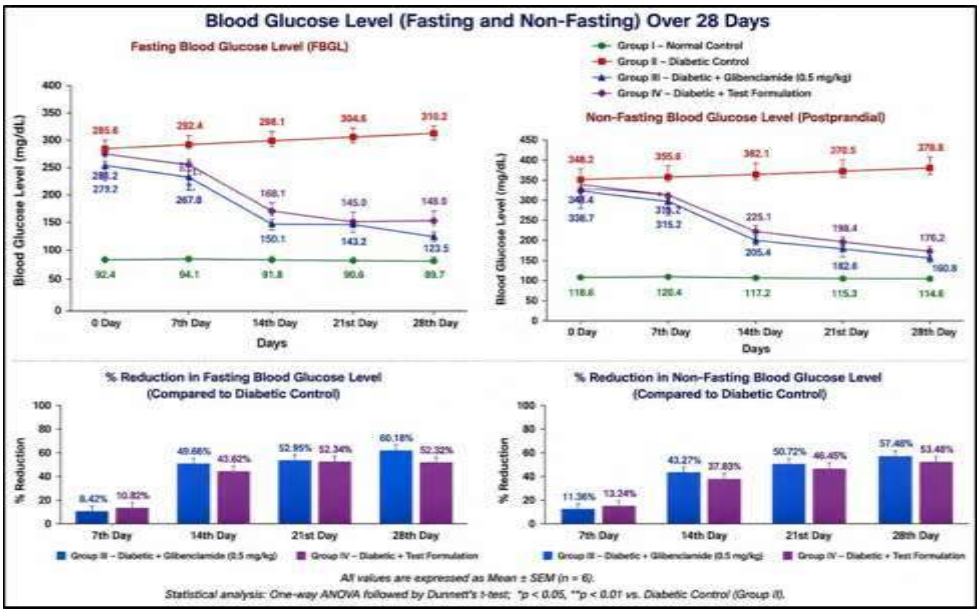

Figure 5 Blood Glucose Level (Fasting and Non-Fasting)

The diabetic control group showed continuously elevated fasting and non-fasting blood glucose levels throughout the study. Treatment with Glibenclamide and the polyherbal formulation significantly reduced blood glucose levels ($p < 0.05$). Although Glibenclamide produced greater antihyperglycemic activity, the formulation also showed effective control of both fasting and postprandial hyperglycemia, indicating promising antidiabetic potential.

Histopathological Study

Histopathological examination of the pancreas and liver revealed significant tissue damage in diabetic control rats. Pancreatic sections showed a marked reduction in the number of islets of Langerhans and β-cells, along with fibrosis and inflammatory cell infiltration. Liver sections exhibited fatty changes, sinusoidal dilation, congestion, and necrosis. Treatment with the polyherbal formulation resulted in considerable recovery of pancreatic and hepatic architecture, with improved β-cell population, reduced inflammation, and near-normal liver histology. The findings were comparable to those observed with Glibenclamide treatment, indicating the protective and antidiabetic potential of the polyherbal formulation.

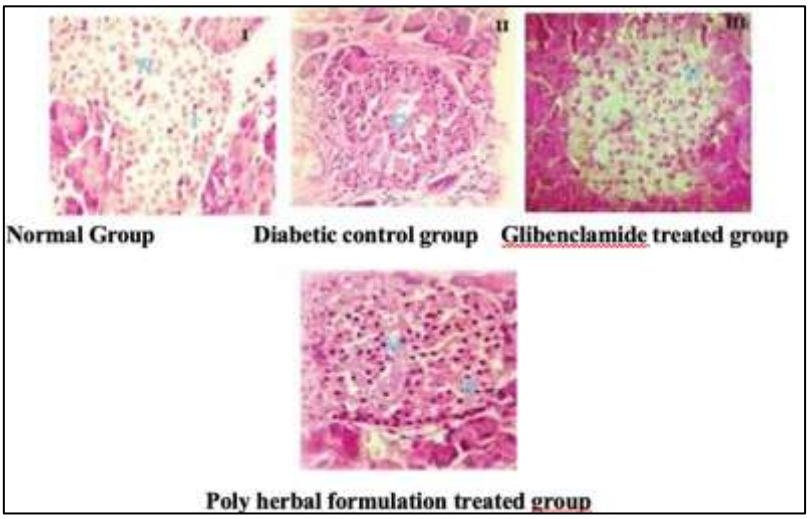

Figure 6: Histopathological Examination of Pancreatic Tissue in Experimental Rats.

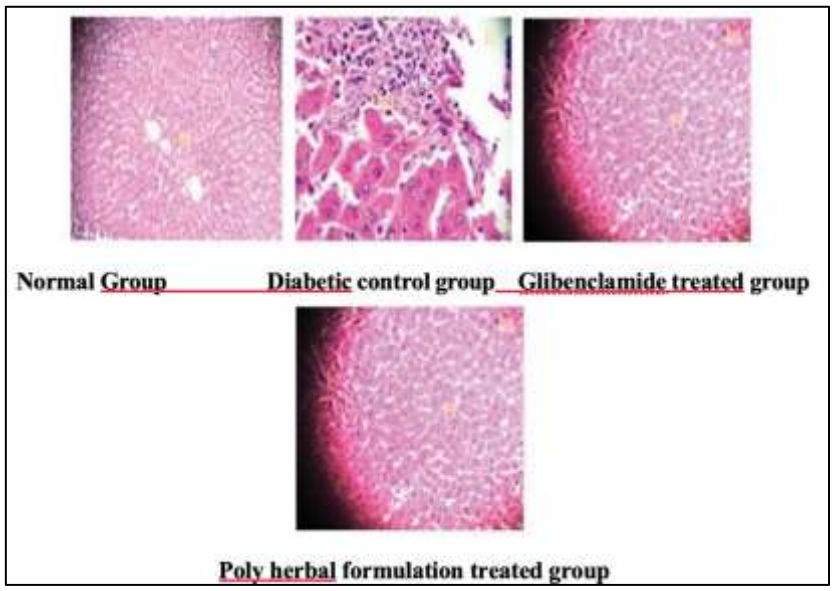

Figure 7: Histopathological Examination of Liver Tissue in Experimental Rats.

CONCLUSIONS

The present study demonstrates that the evaluated formulation possesses significant antidiabetic activity in streptozotocin–nicotinamide induced diabetic rat models. The formulation effectively reduced elevated fasting blood glucose levels over the 28-day treatment period and showed a consistent hypoglycemic effect comparable to the standard drug Glibenclamide. In addition to glucose-lowering activity, the formulation improved body weight and showed a favorable effect on insulin levels, indicating better metabolic control and possible protection or regeneration of pancreatic β-cells. The overall improvement in biochemical parameters suggests restoration of normal glucose homeostasis and reduction of diabetes-associated metabolic disturbances. The observed antidiabetic effect may be attributed to enhanced peripheral glucose utilization, improved insulin sensitivity, and possible antioxidant or cytoprotective mechanisms. These findings support the therapeutic potential of the formulation in the management of diabetes mellitus. However, despite promising results in the experimental model, further detailed studies are necessary. Comprehensive pharmacological investigations, mechanistic studies, long-term toxicity assessments, and clinical trials are required to confirm its safety, efficacy, and applicability in human use.

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