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SYNERGISTIC THERAPEUTIC EFFECTS OF ALANGIUM SALVI FOLIUM, TINOSPORA CORDIFOLIA, TERMINALIA CHEBULA, AND PAVONIA ZEYLANICA IN A POLYHERBAL TABLET FORMULATION

Karthik Gummadavelli[1], Dr. Manish Kumar Mishra[2], Dr. CH. Suresh

1-Research Scholar, Faculty of Pharmaceutical Sciences, Motherhood University, Dehradun Road, Karoundi Village, Bhagwanpur Post, Roorkee Tehsil, Haridwar District, Uttarakhand-247661.

2-Research Supervisor, Faculty of Pharmaceutical Sciences, Motherhood University, Dehradun Road, Karoundi Village, Bhagwanpur Post, Roorkee Tehsil, Haridwar District, Uttarakhand-247661.

3- Research Co-Supervisor, Vijay College of Pharmacy, Borgaon, Nizamabad, Borgaon (Kalan), Telangana 503003.

Abstract:

In Diabetes mellitus, chronic hyperglycemia, insulin failure, dyslipidemia, and inflammation result in substantial microvascular and macrovascular effects. The present work involves the development and evaluation of a polyherbal film-coated tablet prepared from ethanolic extracts of Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula and Pavonia zeylanica for antidiabetic, anti-inflammatory and antihyperlipidemic activities. Phytochemical screening of produced plant extracts of ethanol and formulation in optimal film coated tablet (F8) was performed. The pharmacological effectiveness of the formulation was evaluated in streptozotocin-induced diabetic Wistar rats by assessing fasting blood glucose, serum insulin, HbA1c, oral glucose tolerance, total protein, pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) and serum lipid profile. The optimized formulation exhibited dosage dependent therapeutic efficacy with 500 mg/kg showing similar results as that of Glibenclamide. The formulation significantly reduced fasting blood glucose, HbA1c, TNF-α, IL-6, IL-1β, total cholesterol, LDL cholesterol, triglycerides and VLDL cholesterol and increased serum insulin, HDL cholesterol, protein and glucose tolerance ($p < 0.001$). The pharmacological effects may be attributed to the synergistic impact of flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, and other bioactive phytoconstituents present in the selected medicinal plants. Polyherbal film coated tablet demonstrated strong antidiabetic, anti-inflammatory and antihyperlipidemic actions indicating the safe and efficient herbal remedy for diabetes mellitus and associated metabolic consequences. Toxicology, pharmacokinetics, molecular processes and clinical efficacy studies should validate its therapeutic potential for people.

Keywords: *Diabetes mellitus; Polyherbal formulation; Film-coated tablet; Alangium Salvi folium; Tinospora cordifolia; Terminalia chebula; Pavonia zeylanica; Streptozotocin-induced diabetes; Antidiabetic activity; Anti-inflammatory activity; Antihyperlipidemic activity; Phytoconstituents.*

Corresponding author:

Karthik Gummadavelli,

Research Scholar, Faculty of Pharmaceutical Sciences,
Motherhood University, Dehradun Road, Karoundi Village,
Bhagwanpur Post, Roorkee Tehsil, Haridwar District, Uttarakhand-247661.
Email Id: kartheek100528@gmail.com
Mobile: 96429 78833

QR CODE

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INTRODUCTION:

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. According to the International Diabetes Federation, the global prevalence of diabetes continues to rise, representing a significant public health challenge worldwide. Long-term hyperglycemia is associated with various microvascular and macrovascular complications including nephropathy, neuropathy, retinopathy, cardiovascular diseases, and dyslipidemia, thereby increasing morbidity and mortality among diabetic patients.Type 2 diabetes mellitus (T2DM) accounts for approximately 90–95% of all diabetes cases and is commonly associated with insulin resistance, obesity, sedentary lifestyle, and genetic predisposition. Despite the availability of numerous synthetic antidiabetic agents such as sulfonylureas, biguanides, thiazolidinediones, and insulin preparations, long-term treatment is often associated with adverse effects including hypoglycemia, gastrointestinal disturbances, weight gain, and reduced patient compliance. Therefore, there is an increasing need for safer and more effective alternative therapeutic approaches.

Medicinal plants have been utilized for centuries in traditional healthcare systems due to their therapeutic efficacy and minimal side effects. Plant-derived bioactive compounds such as flavonoids, alkaloids, tannins, terpenoids, glycosides, and phenolic compounds have demonstrated significant antidiabetic, antioxidant, anti-inflammatory, and antihyperlipidemic properties. These phytoconstituents exert their effects through multiple mechanisms including enhancement of insulin secretion, regeneration of pancreatic β-cells, inhibition of carbohydrate metabolizing enzymes, improvement of glucose uptake, and reduction of oxidative stress.Among medicinal plants, Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula, and Pavonia zeylanica have gained considerable attention due to their diverse pharmacological activities. Tinospora cordifolia possesses immunomodulatory, antioxidant, and antidiabetic properties. Terminalia chebula is rich in phenolic compounds and exhibits potent antioxidant and antihyperglycemic effects. Alangium Salvi folium has demonstrated anti-inflammatory and antidiabetic activities, whereas Pavonia zeylanica contains bioactive constituents capable of modulating glucose metabolism and oxidative stress.

Polyherbal formulations represent a promising strategy in herbal therapeutics because they combine multiple medicinal plants to achieve synergistic pharmacological effects. The combined action of various phytoconstituents may enhance therapeutic efficacy while reducing toxicity and improving patient compliance. Furthermore, formulation of herbal extracts into solid dosage forms such as film-coated tablets provide advantages including dose uniformity, stability, ease of administration, and improved patient acceptability. Oxidative stress plays a critical role in the pathogenesis of diabetes and its associated complications. Hyperglycemia-induced generation of reactive oxygen species causes cellular damage, inflammation, and impaired insulin signaling pathways. Therefore, medicinal plants possessing both antioxidant and antidiabetic activities may provide dual therapeutic benefits in the management of diabetes mellitus.In the present investigation, ethanolic extracts of Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula, and Pavonia zeylanica were evaluated for phytochemical composition, antioxidant activity, antidiabetic efficacy, and antihyperlipidemic potential. Bioactive constituents were isolated through chromatographic techniques and subsequently formulated into a polyherbal film-coated tablet. The developed formulation was subjected to comprehensive physicochemical evaluation and pharmacological assessment in experimental animal models to establish its therapeutic potential in diabetes management.

2. MATERIALS AND METHODS:

2.1 Materials

All chemicals and reagents used in the present study were of analytical grade. Petroleum ether, chloroform, ethanol, methanol, Folin–Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), Aluminium chloride, ferric chloride, sodium carbonate, silica gel, and other analytical reagents were procured from standard commercial suppliers. Glibenclamide was used as the reference standard for antidiabetic studies. Microcrystalline cellulose, polyvinyl pyrrolidone (PVP K-30), crospovidone, magnesium stearate, talc, and hydroxypropyl methylcellulose (HPMC) were used for tablet formulation.

2.2 Plant Materials Fresh plant materials including leaves of Alangium Salvi folium, stem of Tinospora cordifolia, fruits of Terminalia chebula, and whole plant of Pavonia zeylanica were collected from authenticated sources. The collected materials were washed thoroughly with distilled water, shade-dried at room temperature, and pulverized using a mechanical grinder. The powdered materials were passed through a suitable sieve and stored in airtight containers until further use.

2.3 Preparation of Plant Extracts

The powdered plant materials were subjected to

Soxhlet extraction using ethanol as extraction solvent. Extraction was continued until complete exhaustion of plant material. The obtained extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extracts were further dried and preserved in desiccators for subsequent studies.

2.4 Experimental Design and Animal Grouping

The antidiabetic activity of the optimized polyherbal film-coated tablet formulation was evaluated in streptozotocin (STZ)-induced diabetic Wistar albino rats. Diabetes was induced by a single intraperitoneal administration of streptozotocin (60 mg/kg body weight). Animals exhibiting fasting blood glucose levels above 250 mg/dL after 72 h of STZ administration were considered diabetic and selected for the study.The animals were randomly divided into five groups comprising six animals in each group. Group I served as the normal control and received distilled water throughout the experimental period. Group II served as the diabetic control and received STZ without any treatment. Group III received the standard antidiabetic drug Glibenclamide (0.25 mg/kg body weight). Groups IV and V received the optimized polyherbal film-coated tablet formulation (F8) at doses of 250 mg/kg and 500 mg/kg body weight, respectively. Treatment was continued for 21 consecutive days.

The experimental grouping was designed to evaluate the dose-dependent antidiabetic, anti-inflammatory, and antihyperlipidemic effects of the developed polyherbal formulation in comparison with the standard drug and untreated diabetic control groups. Various biochemical parameters including fasting blood glucose, serum insulin, glycated hemoglobin (HbA1c), inflammatory cytokines (TNF-α, IL-6, and IL-1β), total protein, oral glucose tolerance, and lipid profile were assessed at predetermined intervals to determine the therapeutic efficacy of the formulation.

Table 1. Experimental Grouping of Animals

Group	Treatment	Dose
Group I	Normal Control	Distilled water
Group II	Diabetic Control	STZ(60 mg/kg, i.p)
Group III	Standard Drug	Glibenclamide(0.25mg/kg)
Group IV	Formulation F8	250 mg/kg
Group V	Formulation F8	500 mg/kg

2.5Experimental Animals

Healthy Wistar albino rats weighing 150–200 g were used for in vivo studies. Animals were housed under standard laboratory conditions with free access to food and water. Experimental procedures were conducted following Institutional Animal Ethics Committee guidelines.

2.6 **Evaluation of Antidiabetic Activity of Isolated Fraction**

The following biochemical parameters were going to analyze to determine the antidiabetic activity:

2.7 Estimation of Cytokines in Serum

Blood samples were allowed to clot for 20–30 min at room temperature and centrifuged at 3000 rpm for 10 min to separate serum, which was stored at −20°C until analysis. Serum cytokine levels were determined using ELISA according to the manufacturer's protocol. Briefly, standards and serum samples (100 µL) were added to antibody-coated 96-well plates, followed by incubation, washing, addition of biotinylated detection antibody and HRP-streptavidin, TMB substrate development, and reaction termination. Absorbance was measured at 450 nm, and cytokine concentrations were calculated from the standard curve and expressed as pg/mL serum (Huang et al., 2020).)

2.8 Determination of Fasting Blood Glucose (FBG) Levels

Rats with fasting blood glucose levels >200 mg/dL along with polyuria and polydipsia were considered diabetic and included in the study. Animals were randomly divided into five groups (n = 6): Group I (normal control, distilled water 10 mL/kg, p.o.), Group II (diabetic control, distilled water 10 mL/kg, p.o.), Group III (Glibenclamide 0.25 mg/kg, p.o.), Group IV (optimized formulation 250 mg/kg, p.o.), and Group V (optimized formulation 500 mg/kg, p.o.). Treatments were administered orally once daily for 30 days. Fasting blood glucose levels were recorded on days 1, 10, 20, and 30. At the end of the study, animals were euthanized with thiopentone sodium (75 mg/kg, i.p.), and blood and tissue samples were collected for biochemical analysis (Szkudelski, 2001)

2.9. **Estimation of Plasma Insulin by the Rat Diaphragm Method**

Rats were fasted for 24 h, sacrificed, and the diaphragms were excised, divided into hemidiaphragms, and maintained in cold balanced

salt solution (BSS). Each hemidiaphragm was incubated in glucose-BSS with or without insulin at 37°C for 90 min under 95% O_2 and 5% CO_2. After incubation, tissues were washed, dried, weighed, and glucose uptake was determined based on tissue dry weight. The increase in glucose uptake compared to the control was considered proportional to insulin activity (Ghosh, 1984).

2.10 Estimation of Glycated Hemoglobin (HbA1c)

Diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg) prepared in 0.1 M citrate buffer (pH 4.5), while control rats received citrate buffer alone. Blood samples were collected from the tail vein, and HbA1c was determined using an automated ion-exchange HPLC analyzer after hemolysis of whole blood collected in EDTA tubes.

2.11 Estimation of Total Protein (Lowry Method)

Serum was separated by centrifugation at 3000 rpm for 10 min and stored at −20°C until analysis. Total serum protein was estimated by the Lowry method using alkaline copper reagent and Folin–Ciocalteu reagent. Absorbance was measured at 660 nm, and protein concentration was calculated from a bovine serum albumin (BSA) standard curve and expressed as g/dL or mg/mL serum (Lowry et al., 1951).

2.12 Oral Glucose Tolerance Test (OGTT) in Diabetic Rats:

The oral glucose tolerance test (OGTT) was performed after overnight fasting (12 h). Following measurement of baseline fasting blood glucose, rats received an oral glucose load (2 g/kg). Blood glucose levels were measured from the tail vein at 30, 60, 90, and 120 min using a digital glucometer to evaluate glucose tolerance and the antihyperglycemic effect of the test formulation (Sharma et al., 2010).

2.2 ANTIHYPERLIPIDEMIC STUDY

Rats were housed under standard laboratory conditions (25 ± 2°C, 55 ± 10% relative humidity, 12 h light/dark cycle) with free access to pellet diet and water. Hyperlipidemia was induced by administering 25% D-fructose in drinking water for 21 days. Thirty rats were randomly divided into five groups (n = 6). Serum total cholesterol was estimated using the enzymatic CHOD–PAP method by incubating serum with cholesterol reagent at 37°C for 5–10 min, measuring absorbance at 505 nm, and calculating the concentration from a standard curve. Results were expressed as mg/dL serum (Allain et al., 1974; Pesce & Bodourian, 1976).

Table 2: Animal Grouping

Group	Treatment
Group I	Normal control (received distilled water)
Group II	Negative control (25% D-fructose in drinking water)
Group III	Standard group (25% D-fructose + Fenofibrate 250 mg/kg p.o.)
Group IV	Test group I (25% D-fructose + Test fraction 200 mg/kg p.o.)
Group V	Test group II (25% D-fructose + Test fraction 300 mg/kg p.o.)

Group I animals received only distilled water and served as the normal control. Groups II– V were provided with 25% D-fructose in drinking water throughout the experimental period. The standard drug fenofibrate (250 mg/kg, p.o.) was administered to Group III animals. The test groups (Group IV and Group V) received the isolated fraction (Selected Formulation) at doses of 200 mg/kg and 300 mg/kg orally, respectively, during the 21-day treatment period (Lad & Kolhe, 2023).

2.2.1 Evaluation Parameters for hyperlipidemia

At the end of the experimental period, the animals were fasted overnight and blood samples were collected under light anesthesia. The blood samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to obtain serum. The separated serum samples were stored at −20 °C until biochemical analysis.

2.2.2 Estimation of Total Cholesterol (TC)
Serum total cholesterol (TC) was estimated using the enzymatic CHOD–PAP method with a commercial diagnostic kit according to the manufacturer's instructions. After incubation of the serum sample with the working reagent at 37°C for 5–10 min, absorbance was measured at 505 nm using a UV–Visible spectrophotometer. Total cholesterol concentration was calculated from a standard curve and expressed as mg/dL of serum (Allain et al., 1974; Pesce & Bodourian, 1976)

2.2.3. **Estimation of Serum Triglycerides (TG)**
To measure blood triglyceride (TG) levels, commercially available diagnostic kits were used using the GPO-PAP enzymatic colorimetric technique, following manufacturer's instructions. Lipase hydrolyzes serum triglycerides to generate glycerol and free fatty acids. Glycerol kinase phosphorylates glycerol to make glycerol-3-phosphate, which oxidizes to hydrogen peroxide. With peroxidase, hydrogen peroxide combines with chromogenic substrates such 4- amino antipyrine and phenol to form a Quinoneimine complex. Triglyceride concentration determines color intensity (Fossati & Prencipe, 1982).

2.2.4 Estimation of High-Density Lipoprotein Cholesterol (HDL-C)
Serum HDL-C was tested using commercial precipitation–enzymatic colorimetric kits. The method precipitates LDL and VLDL from blood using phosphotungstic acid and magnesium chloride. Centrifugation separates clear HDL-cholesterol supernatant. Then, enzymatic CHOD-PAP hydrolyzes cholesterol esters to free cholesterol to test the supernatant's cholesterol. From free cholesterol, cholesterol oxidase creates hydrogen peroxide. Peroxidase and hydrogen peroxide generate a Quinoneimine-colored complex with phenol and 4-aminoantipyrine. Burstein et al. (1970) found that HDL-cholesterol concentration influences color intensity. At 505 nm, a UV–Visible spectrophotometer measured the color's absorbance against a reagent blank. HDL-cholesterol was measured in mg/dL using a standard calibration curve.

2.2.5 Estimation of Low-Density Lipoprotein Cholesterol (LDL-C) and very low- density lipoprotein cholesterol (VLDL-C)
Serum LDL-C was indirectly determined using the Fried Ewald equation, which is extensively used to calculate LDL cholesterol from other lipid profile factors. This approach begins with enzymatic colorimetric measurements of total cholesterol (TC), triglycerides (TG), and HDL-C. Triglycerides are used to determine VLDL-C
The LDL-C concentration was calculated using the following formula:

LDL-C=TC-(HCL-C+VLDL-C) and VLDL-C=TG/5

The calculated LDL-C values were expressed as mg/dL of serum. This formula is valid when triglyceride levels are below 400 mg/dL and has been widely used for lipid profile estimation in experimental and clinical studies (Fried Ewald et al., 1972).

Statistical Analysis
The experimental data were reported as mean ± SEM for each group (n = 6). Statistical significance between experimental groups was determined by one-way analysis of variance (ANOVA). The treatment and control groups were compared for significant differences using Dunnett's post hoc test. Student's t-test was used to compare two groups as needed. Statistics were calculated using relevant tools. The level of significance was $p<0.05$, with $p<0.01$ and $p<0.001$ being very significant.
The results were presented in tables and graphs for clarity.

3.RESULTS AND DISCUSSION:

3.1IN-VIVO ANTIDIABETIC ACTIVITY

To establish the antidiabetic efficacy of the optimized polyherbal film-coated tablet formulation (F8), a comprehensive pharmacological evaluation was performed in streptozotocin-induced diabetic rats. Diabetes induction resulted in significant alterations in inflammatory cytokines, blood glucose levels, insulin secretion, glycated hemoglobin, oral glucose tolerance, serum protein concentration, and lipid profile parameters. Administration of formulation F8 at two different dose levels (250 and 500 mg/kg) produced significant improvements in these biochemical markers when compared with the diabetic control group. The observed effects were compared with the standard antidiabetic drug Glibenclamide to assess the therapeutic potential of the developed formulation. The results obtained from the study are discussed under the following sections.

3.1.1 Evaluation of Cytokines

Table 3: Effect of Formulation (F8) on Serum Cytokine Levels in STZ-Induced Diabetic Rats

Group / Treatment	TNF-α(pg/mL)	IL-6 (pg/mL)	IL-1β(pg/mL)
Group-I–Normal control (Distilled water)	22.5 ± 1.8	18.7 ± 1.5	15.4 ± 1.2
Group II – Diabeticcontro (STZ 60 mg/kg i.p.-induced)	78.9 ± 4.2	65.3 ± 3.7	58.6 ± 3.1
Group III Standard drug (Glibenclamide 0.25 mg/kg)	28.4 ± 2.1	25.6 ± 2.0	22.1 ± 1.8
Group IV – F8 Film- coated tablet (250 mg/kg)	46.2 ±3.0	38.9 ± 2.6	35.7 ± 2.4
Group V – F8	32.8 ± 2.4	29.1 ± 2.1	26.2 ± 1.9
Film-coated tablet (500 mg/kg)			

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats showed a significant ($p<0.001$) elevation in serum TNF-α, IL-6, and IL-1β levels compared to the normal control group. Treatment with Glibenclamide significantly reduced all inflammatory cytokines. Similarly, the F8 film-coated tablet produced a dose-dependent reduction in TNF-α, IL-6, and IL-1β levels, with the 500 mg/kg dose showing greater anti-inflammatory activity than the 250 mg/kg dose and approaching the effect of the standard drug.

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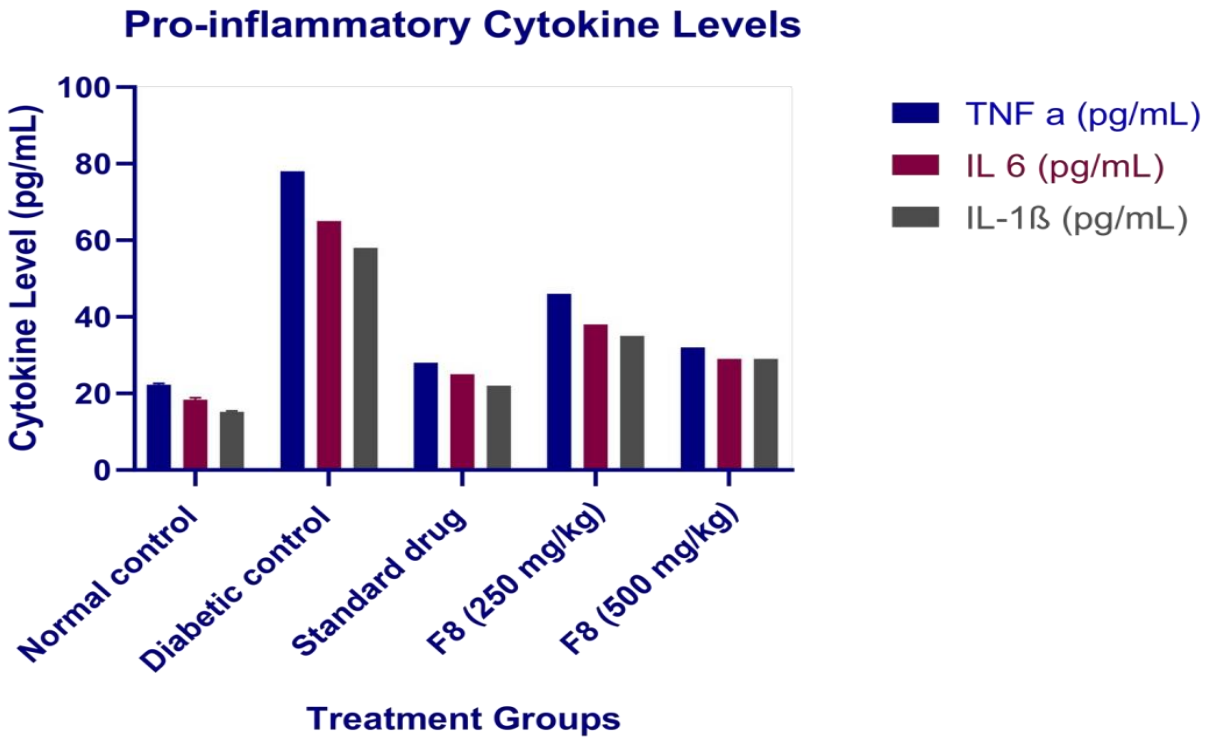

Figure 1: Effect of Formulation (F8) on Serum Cytokine Levels in STZ-Induced Diabetic Rats

Source: *Researcher's Data Analysis,2024*

Figure illustrates the effect of formulation F8 on serum pro-inflammatory cytokine levels in streptozotocin-induced diabetic rats. The diabetic control group showed a significant increase in TNF-α, IL-6, and IL-1β levels compared with the normal control group, indicating enhanced systemic inflammation. Treatment with Glibenclamide significantly suppressed these inflammatory markers. Similarly, F8 film-coated tablets produced a significant dose-dependent reduction in cytokine levels, with the 500 mg/kg dose demonstrating greater anti-inflammatory activity than the 250 mg/kg dose and restoring cytokine levels closer to those of the normal control group. These findings indicate that formulation F8 effectively attenuates diabetes-induced inflammation.

3.1.2 Analysis of Blood Glucose Level

Table 4: Effect of Formulation (F8) on Blood Glucose Level in STZ-Induced Diabetic Rats

Groups	Day 0 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Group I – Normal Control (Distilled Water)	110.80 ± 3.80	108.89 ± 2.80	111.64 ± 3.50	114.00 ± 2.16
Group II – Diabetic Control (STZ 60 mg/kg, i.p.)	340.20 ± 6.73	258.20 ± 5.00	259.20 ± 8.20	269.00 ± 11.00
Group III – Standard Drug (Glibenclamide 0.25 mg/kg)	343.60 ± 40.80	256.81 ± 41.30	193.82 ± 15.13	138.00 ± 3.70
Group IV – F8 Film-Coated Tablet (250 mg/kg)	349.80 ± 43.10	209.20 ± 28.80	134.00 ± 2.14	120.60 ± 2.10
Group V – F8 Film-Coated Tablet (500 mg/kg)	390.80 ± 52.00	197.40 ± 29.40	132.80 ± 2.60	120.00 ± 2.60

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) exhibited a significant ($p<0.001$) increase in blood glucose levels compared to the normal control group throughout the study. Treatment with Glibenclamide (0.25 mg/kg) markedly reduced blood glucose levels, confirming its antihyperglycemic effect. Similarly, the F8 film-coated tablet significantly lowered blood glucose in a dose-dependent manner, with the 500 mg/kg dose producing a slightly greater reduction than the 250 mg/kg dose. By Day 21, both F8-treated groups showed blood glucose levels close to those of the standard treatment, indicating effective glycemic control.

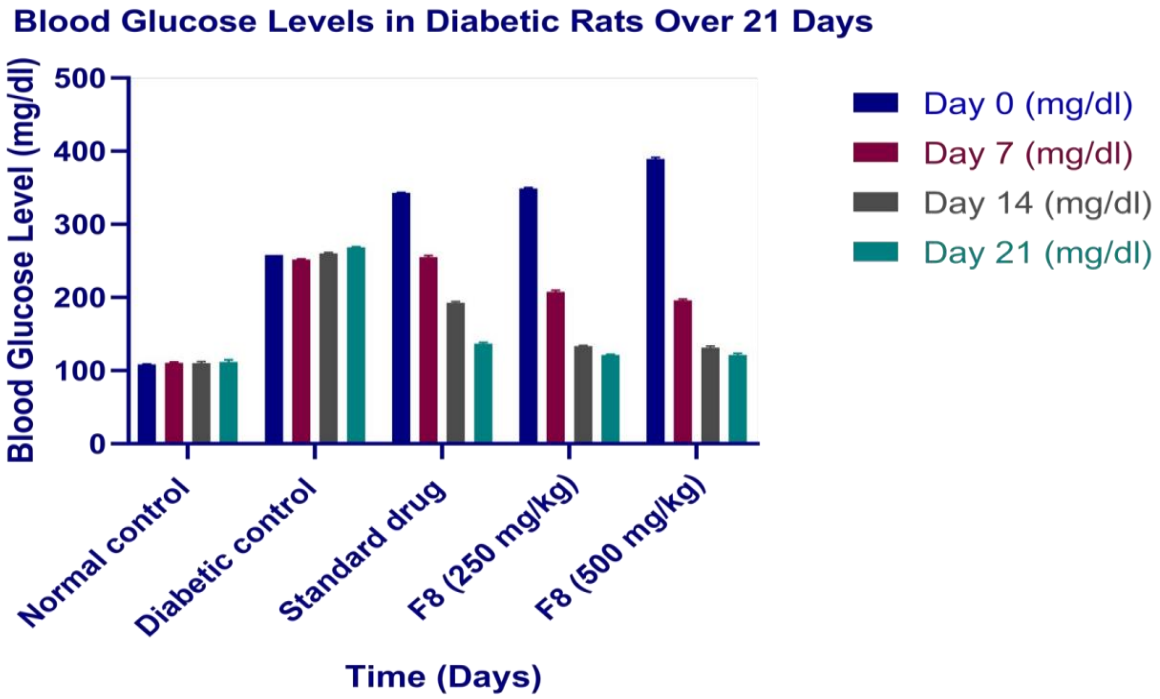

Figure2: Effect of Formulation (F8) on Blood Glucose Levels in STZ-Induced Diabetic Rats

Source: *Researcher's Data Analysis,2024*

The above figure shows the effect of formulation F8 on blood glucose levels in STZ-induced diabetic rats. The diabetic control group maintained significantly elevated blood glucose levels throughout the study, confirming persistent hyperglycemia. In contrast, treatment with Glibenclamide and F8 film-coated tablets significantly reduced blood glucose levels in a time- and dose-dependent manner. The 500 mg/kg dose of F8 produced a greater reduction than the 250 mg/kg dose, with both treatment groups showing glucose levels approaching normal by Day 21, indicating effective antihyperglycemic activity.

3.1.3 **Analysis of serum Insulin level**

Table 5: Effect of Formulation (F8) on serum Insulin level in STZ-Induced Diabetic Rats

Group Treatment (n = 6)	Serum Insulin (µU/ml)
Group I – Normal control (Distilled water)	18.16 ± 0.55
Group II – Diabetic control (STZ 60 mg/kg, i.p.-induced)	7.13 ± 0.31
Group III – Standard drug (Glibenclamide 0.25 mg/kg)1	16.66 ± 0.33
Group IV – F8 Film-coated tablet (250 mg/kg)	13.33 ± 0.33
Group V – F8 Film-coated tablet (500 mg/kg)	15.33 ± 0.33

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) reduction in serum insulin levels compared to the normal control group. Treatment with Glibenclamide significantly restored insulin levels. Similarly, the F8 film-coated tablet increased serum insulin concentrations in a dose-dependent manner, with the 500 mg/kg dose producing a greater effect than the 250 mg/kg dose and approaching the response of the standard drug. These results indicate that F8 effectively improved insulin secretion in diabetic rats.

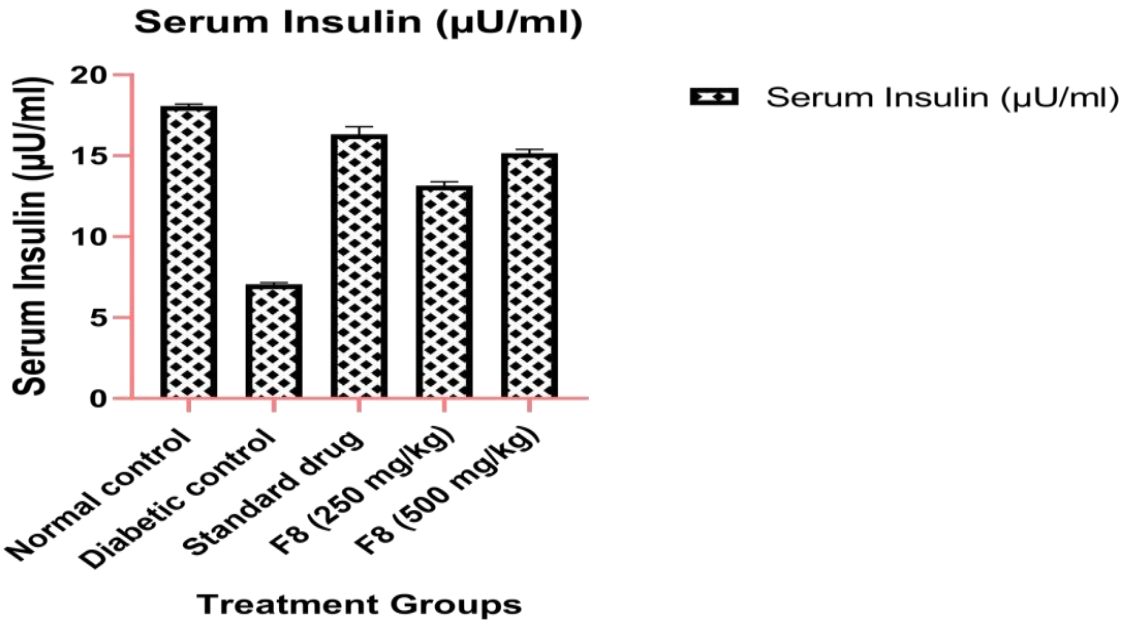

Figure 3: Effect of Formulation (F8) on serum Insulin level in STZ-Induced Diabetic Rats

Source: *Researcher's Data Analysis,2024*

The figure illustrates the effect of formulation F8 on serum insulin levels in STZ-induced diabetic rats. The diabetic control group showed a marked reduction in serum insulin levels compared to the normal control group, indicating impaired pancreatic β-cell function. Treatment with Glibenclamide significantly restored insulin levels. Likewise, F8 film-coated tablets increased serum insulin concentrations in a dose-dependent manner, with the 500 mg/kg dose producing a greater improvement than the 250 mg/kg dose and showing an effect comparable to the standard treatment.

3.1.4 Analysis of Serum HbA1c level

Table6: Effect of Formulation (F8) on Serum HbA1c level in STZ-Induced Diabetic Rats

Groups	Glycosylated Hemoglobin (%HbA1c)
Group I – Normal control (Distilled water)	6.24 ± 0.38
Group II – Diabetic control (STZ 60 mg/kg, i.p.-induced)	12.36 ± 0.91
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	6.95 ± 0.42
Group IV – F8 Film-coated tablet (250mg/kg)	6.72 ± 0.42
Group V – F8 Film-coated tablet (500 mg/kg)	6.31 ± 0.40

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) increase in HbA1c levels compared to the normal control group. Treatment with Glibenclamide significantly reduced HbA1c, indicating improved long-term glycemic control. Similarly, the F8 film-coated tablet produced a dose-dependent reduction in HbA1c levels, with the 500 mg/kg dose showing greater efficacy than the 250 mg/kg dose and restoring HbA1c values close to those of the normal control group

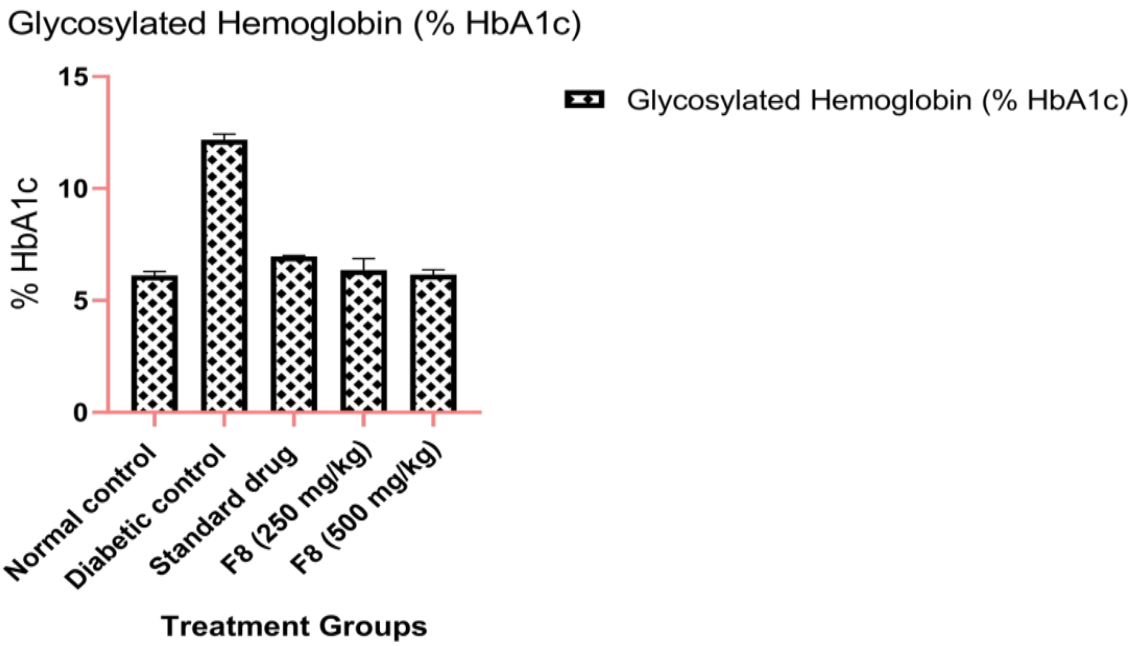

Figure 4: Effect of Formulation (F8) on Serum HbA1c level in STZ-Induced Diabetic Rats

Source: *Researcher's Data Analysis,2024*

The above figure illustrates the effect of formulation F8 on HbA1c levels in STZ-induced diabetic rats. The diabetic control group exhibited significantly elevated HbA1c levels compared to the normal control group, indicating poor long-term glycemic control. Treatment with Glibenclamide markedly reduced HbA1c levels. Likewise, F8 film-coated tablets produced a significant dose-dependent decrease in HbA1c, with the 500 mg/kg dose showing greater efficacy than the 250 mg/kg dose and restoring HbA1c levels close to those of the normal control group

3.1.5 **Estimation of Total Protein**

Table 7: Effect of Formulation (F8) on Total Protein (g/dL) level in STZ-Induced Diabetic Rats

Groups	Total Protein(g/dL)
Group I – Normal control (Distilled water)	6.82 ± 0.32
Group II – Diabetic control (STZ 60 mg/kg, i.p. induced)	4.21 ± 0.28
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	6.11 ± 0.30
Group IV – F8 Film-coated tablet (250 mg/kg)	5.78 ± 0.26
Group V – F8 Film-coated tablet (500 mg/kg)	6.34 ± 0.29

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) reduction in serum total protein levels compared to the normal control group. Treatment with Glibenclamide significantly restored total protein levels. Similarly, the F8 film-coated tablet increased serum total protein concentrations in a dose-dependent manner, with the 500 mg/kg dose producing a greater improvement than the 250 mg/kg dose and showing an effect comparable to the standard treatment. These findings indicate that F8 effectively improved protein metabolism in diabetic
Rats.

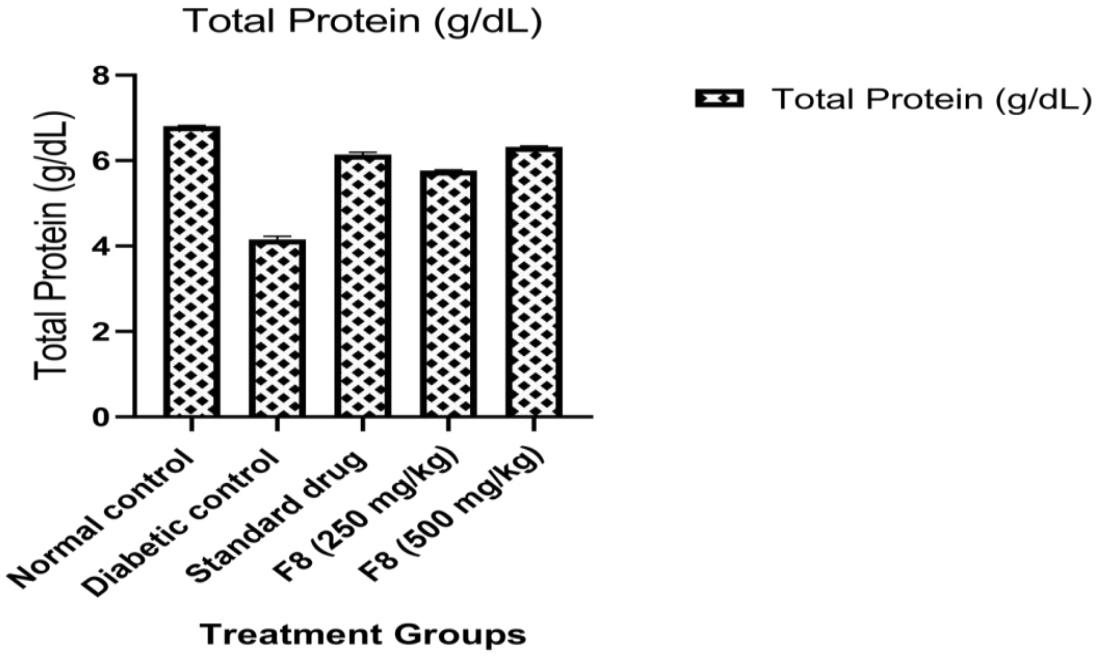

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Figure 5: Effect of Formulation (F8) on Total Protein (g/dL) level in STZ-Induced Diabetic Rats

Source: *Researcher's Data Analysis,2024*

the above figure illustrates the effect of formulation F8 on serum total protein levels in STZ-induced diabetic rats. The diabetic control group showed a significant reduction in total protein levels compared to the normal control group, indicating impaired protein metabolism. Treatment with Glibenclamide significantly restored serum total protein levels. Similarly, F8 film-coated tablets produced a dose-dependent increase in total protein concentration, with the 500 mg/kg dose showing greater improvement than the 250 mg/kg dose and restoring protein levels close to those of the normal control group.

3.1.6 Determination of Oral Glucose Tolerance Test (OGTT)

Table 8: Determination of Oral Glucose Tolerance Test (OGTT)

Groups	0 min (mg/dL)	30 min (mg/dL)	60 min (mg/dL)	120 min (mg/dL)	180 min (mg/dL)
Group I – Normal Control (Distilled water)	88 ± 6.96	193 ± 12.08	182 ± 8.16	100 ± 9.70	93 ± 2.77
Group II – Diabetic Control (STZ 60 mg/kg, i.p.)	245 ± 12.3	365 ± 18.4	352 ± 16.8	310 ± 14.6	286 ± 12.5
Group III – Standard Drug (Glibenclamide 0.25 mg/kg)	90 ± 4.80	175 ± 10.59	159 ± 5.66	91 ± 4.07	85 ± 2.99
Group IV – F8 Film-coated Tablet (250 mg/kg)	88 ± 5.40	161 ± 14.12	135 ± 10.02	89 ± 6.50	89 ± 3.52
Group V – F8 Film-coated Tablet (500 mg/kg)	91 ± 4.51	167 ± 12.28	160 ± 6.94	110 ± 3.80	96 ± 2.49

Source: *Researcher's Data Analysis,2024*

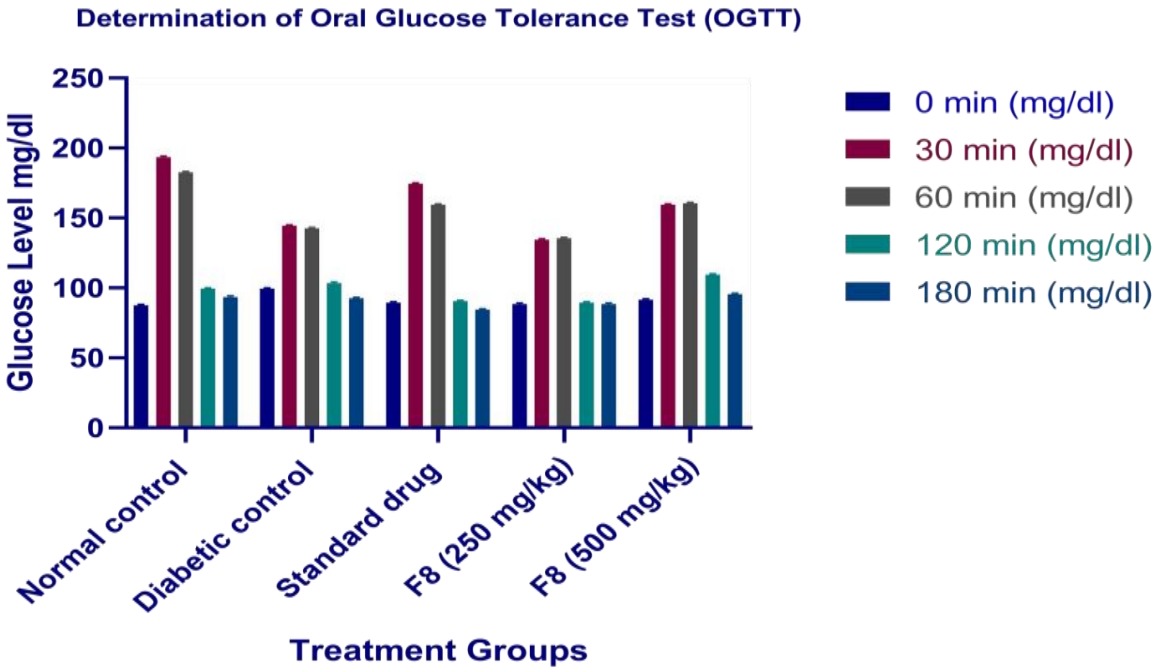

Figure 6: Determination of Oral Glucose

In the oral glucose tolerance test, the normal control group showed a transient increase in blood glucose levels after glucose administration, followed by a gradual return to baseline values. The diabetic control group exhibited impaired glucose tolerance, with persistently elevated blood glucose levels throughout the study. Treatment with Glibenclamide significantly improved glucose tolerance by accelerating the decline in blood glucose levels. Similarly, F8 film-coated tablets produced a dose-dependent improvement in glucose tolerance, with the 500 mg/kg dose showing greater antihyperglycemic activity than the 250 mg/kg dose. By 180 min, the treated groups exhibited blood glucose levels approaching those of the normal control group, indicating effective regulation of glucose homeostasis.

Effect of formulation F8 on oral glucose tolerance in rats was shown in Figure. The OGTT measures the ability of the body to regulate blood glucose levels after glucose administration over a period of time. The normal control group showed a moderate rise in blood glucose levels at 30 minutes followed by a gradual decline towards baseline levels at 120 and 180 minutes, indicating normal glucose metabolism. The diabetic control group exhibited altered glucose tolerance with relatively higher glucose levels and slower decline, indicating impaired glucose utilization.

Tolerance Test (OGTT)
Source: *Researcher's Data Analysis,2024*
Treatment with the standard drug (Glibenclamide 0.25 mg/kg) showed a significant improvement in glucose tolerance, with a faster reduction in blood glucose levels after 60 minutes. Similarly, treatment with F8 film-coated tablets at doses of 250 mg/kg and 500 mg/kg demonstrated improved glucose tolerance. The 250 mg/kg dose showed better regulation with a consistent decline in glucose levels, while the 500 mg/kg dose also exhibited improvement, though with slight variation at later time points.

3.2 ANTIHYPERLIPIDEMIC ACTIVITY

The antihyperlipidemic activity of the formulated herbal tablets was evaluated in streptozotocin-induced diabetic Wistar albino rats by analyzing the lipid profile parameters. Diabetes mellitus is often associated with abnormalities in lipid metabolism, which leads to elevated levels of cholesterol and triglycerides in the blood. Therefore, the effect of the herbal tablet formulation on lipid profile parameters was studied.

The lipid profile parameters evaluated included total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and very low-density lipoprotein cholesterol (VLDL) in experimental animals.

3.2.1 Total Cholesterol Level

Table 9: Effect on Total Cholesterol (mg/dl)

Groups	Total Cholesterol (mg/dl)
Group I – Normal control (Distilled water)	65.82 ± 0.79
Group II – Diabetic control (STZ 60 mg/kg, i.p.-induced)	96.40 ± 0.74
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	70.12 ± 0.68
Group IV – F8 Film-coated tablet (250 mg/kg)	78.05 ± 0.63
Group V – F8 Film-coated tablet (500 mg/kg)	74.26 ± 0.59

Source: *Researcher's Data Analysis,2024*
Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) increase in serum total cholesterol levels compared to the normal control group. Treatment with Glibenclamide significantly reduced total cholesterol levels. Similarly, the F8 film-coated tablet produced a dose-dependent reduction in serum total cholesterol, with the 500 mg/kg dose showing greater antihyperlipidemic activity than the 250 mg/kg dose and an effect comparable to the standard treatment.

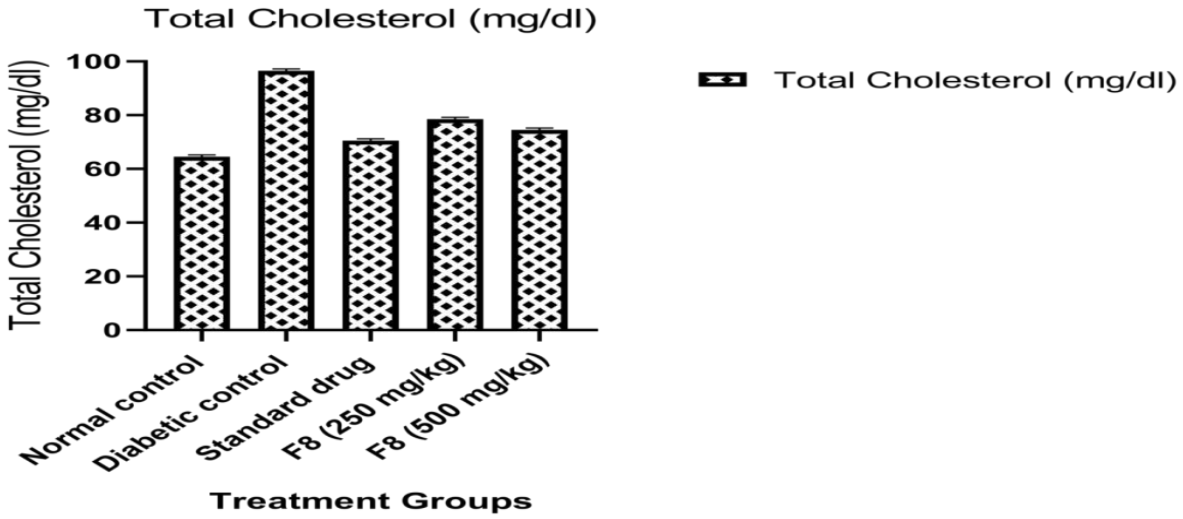

Figure 7: Effect on Total Cholesterol (mg/dl) Level
Source: *Researcher's Data Analysis,2024*

The above figure illustrates the effect of formulation F8 on serum total cholesterol levels in STZ-induced diabetic rats. The diabetic control group showed significantly elevated total cholesterol levels compared to the normal control group, indicating altered lipid metabolism. Treatment with Glibenclamide significantly reduced serum cholesterol levels. Similarly, F8 film-coated tablets produced a dose-dependent reduction in total cholesterol, with the 500 mg/kg dose showing greater hypolipidemic activity than the 250 mg/kg dose and approaching the effect of the standard treatment.

3.2.2 LDL Cholesterol Level

Table 10: Effect on LDL Cholesterol (mg/dl)

Groups	LDL (mg/dl)
Group I – Normal control (Distilled water)	23.10 ± 0.82
Group II – Diabetic control (STZ 60 mg/kg, i.p.-induced)	96.85 ± 0.52
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	36.45 ± 0.71
Group IV – F8 Film-coated tablet (250 mg/kg)	47.88 ± 0.46
Group V – F8 Film-coated tablet (500 mg/kg)	39.12 ± 0.73

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) increase in serum LDL levels compared to the normal control group. Treatment with Glibenclamide significantly reduced LDL levels. Similarly, the F8 film-coated tablet produced a dose-dependent reduction in serum LDL, with the 500 mg/kg dose showing greater efficacy than the 250 mg/kg dose and an effect comparable to the standard treatment, indicating significant antihyperlipidemic activity.

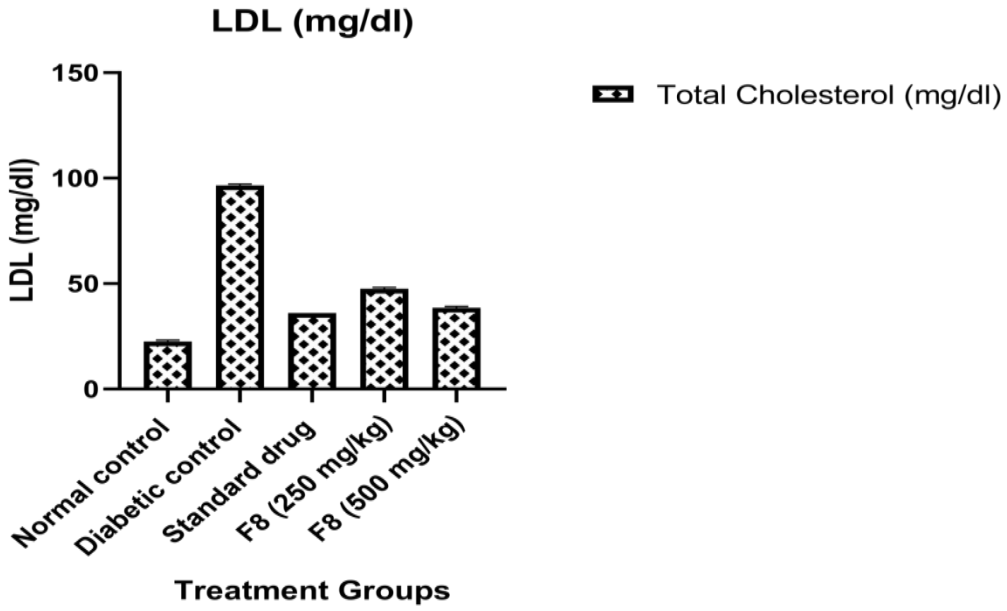

Figure 8: Effect on LDL (mg/dl) level Source: *Researcher's Data Analysis,2024*

The above figure illustrates the effect of formulation F8 on serum LDL cholesterol levels in STZ-induced diabetic rats. The diabetic control group showed significantly elevated LDL levels compared to the normal control group, indicating diabetes-associated dyslipidemia. Treatment with Glibenclamide significantly reduced LDL cholesterol levels. Likewise, F8 film-coated tablets produced a dose-dependent reduction in LDL, with the 500 mg/kg dose showing greater hypolipidemic activity than the 250 mg/kg dose and approaching the effect of the standard treatment.

3.2.3 HDL Cholesterol Level

Table 11: Effect on HDL Cholesterol (mg/dl)

Groups	HDL (mg/dl)
Group I – Normal control (Distilled water)	11.20 ± 0.34
Group II – Diabetic control (STZ 60 mg/kg, i.p.-induced)	5.65 ± 0.295
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	10.72 ± 0.44
Group IV – F8 Film-coated tablet (250 mg/kg)	8.54 ± 0.38
Group V – F8 Film-coated tablet (500 mg/kg)	9.26 ± 0.31

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) reduction in serum HDL levels compared to the normal control group. Treatment with Glibenclamide significantly increased HDL levels. Similarly, the F8 film-coated tablet produced a dose-dependent increase in serum HDL, with the 500 mg/kg dose showing greater improvement than the 250 mg/kg dose and an effect comparable to the standard treatment, indicating significant antihyperlipidemic activity.

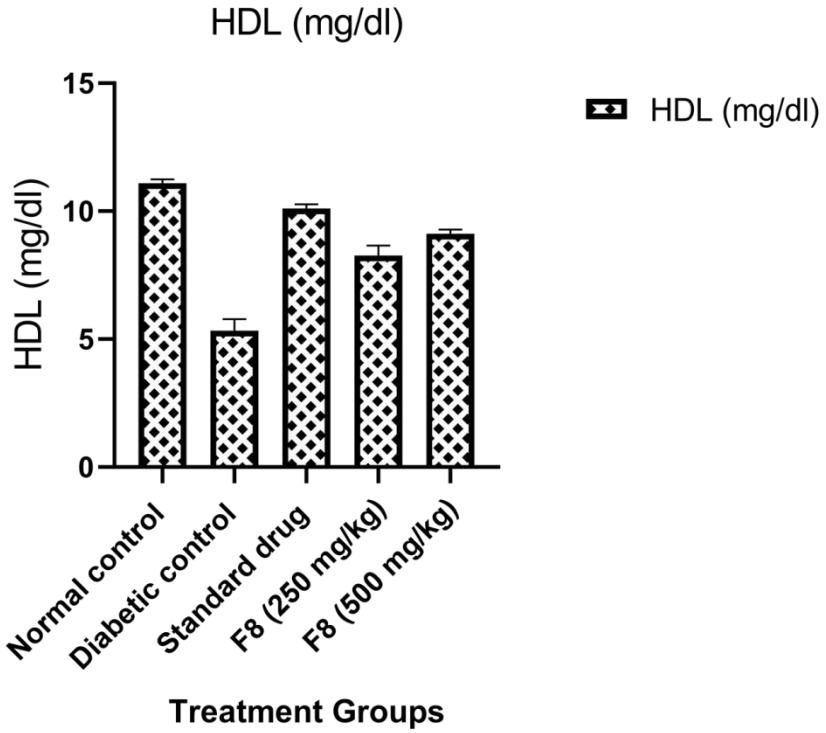

Figure 9: Effect on HDL (mg/dl) level

Source: *Researcher's Data Analysis,2024*

Effect of formulation F8 on HDL cholesterol levels in STZ-induced diabetic rats was shown in Figure 4.36. The diabetic control group (STZ 60 mg/kg, i.p.) showed a significant decrease in HDL cholesterol levels (5.65 ± 0.29 mg/dl) as compared to the normal control group (11.20 ± 0.34 mg/dl), indicating impaired lipid metabolism under diabetic conditions. Treatment with the standard drug (Glibenclamide 0.25 mg/kg) resulted in a significant increase in HDL levels (10.72 ± 0.44 mg/dl), approaching normal values. Similarly, treatment with F8 film-coated tablets at doses of 250 mg/kg and 500 mg/kg showed a notable improvement in HDL cholesterol levels.

The higher dose (500 mg/kg) exhibited a greater increase (9.26 ± 0.31 mg/dl) compared to the lower dose (250 mg/kg) (8.54 ± 0.38 mg/dl), indicating dose-dependent enhancement of HDL levels. A comparative graphical representation of HDL cholesterol levels among different groups is shown in Figure 4.36, demonstrating the potential of formulation F8 in improving the lipid profile in diabetic rats.

3.2.4 VLDL Cholesterol Level

Table 12: Effect on VLDL Cholesterol (mg/dl)

Groups	VLDL (mg/dl)
Group I – Normal control (Distilled water)	17.20 ± 0.62
Group II – Diabetic control (STZ 60 mg/kg, i.p.-induced)	26.48 ± 0.33
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	18.42 ± 0.28
Group IV – F8 Film-coated tablet (250 mg/kg)	21.96 ± 0.24
Group V – F8 Film-coated tablet (500 mg/kg)	19.31 ± 0.19

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) increase in serum VLDL levels compared to the normal control group. Treatment with Glibenclamide significantly reduced VLDL levels. Similarly, the F8 film-coated tablet produced a dose-dependent reduction in serum VLDL, with the 500 mg/kg dose showing greater efficacy than the 250 mg/kg dose and an effect comparable to the standard treatment, indicating significant hypolipidemic activity.

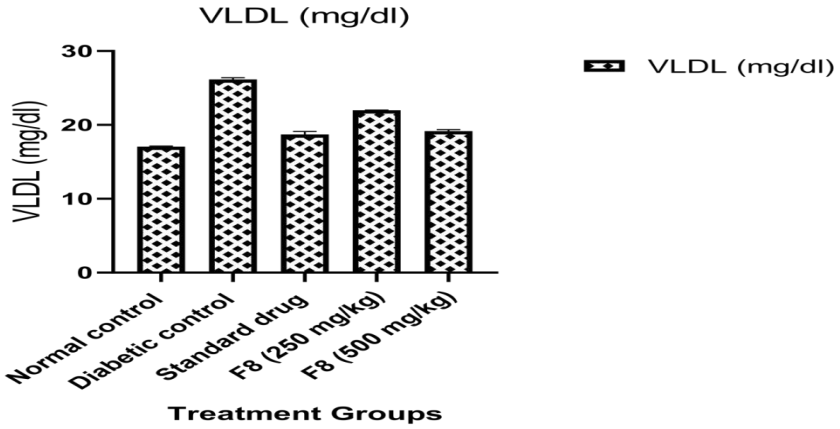

Figure 10: Effect on VLDL (mg/dl) level Source: *Researcher's Data Analysis,2024*

The above figure illustrates the effect of formulation F8 on serum VLDL cholesterol levels in STZ-induced diabetic rats. The diabetic control group showed significantly elevated VLDL levels compared to the normal control group, indicating disturbed lipid metabolism. Treatment with Glibenclamide significantly reduced VLDL levels. Likewise, F8 film-coated tablets produced a dose-dependent reduction in serum VLDL, with the 500 mg/kg dose showing greater hypolipidemic activity than the 250 mg/kg dose and approaching the effect of the standard treatment.

3.2.5 Triglycerides Level

Table 13: Effect on Triglycerides (mg/dl)

Groups	Triglycerides (mg/dl)
Group I – Normal control (Distilled water)	66.72 ± 0.81
Group II – Diabetic control (STZ 60 mg/kg, i.pinduced)	113.60 ± 1.39
Group III – Standard drug (Glibenclamide 0.25 mg/kg)	77.12 ± 0.72
Group IV – F8 Film-coated tablet (250 mg/kg)	83.26 ± 0.95
Group V – F8 Film-coated tablet (500 mg/kg)	78.14 ± 0.87

Source: *Researcher's Data Analysis,2024*

Streptozotocin-induced diabetic rats (Group II) showed a significant ($p<0.001$) increase in serum triglyceride levels compared to the normal control group. Treatment with Glibenclamide significantly reduced triglyceride levels. Similarly, the F8 film-coated tablet produced a dose-dependent reduction in serum triglycerides, with the 500 mg/kg dose showing greater efficacy than the 250 mg/kg dose and an effect comparable to the standard treatment, indicating significant antihyperlipidemic activity.

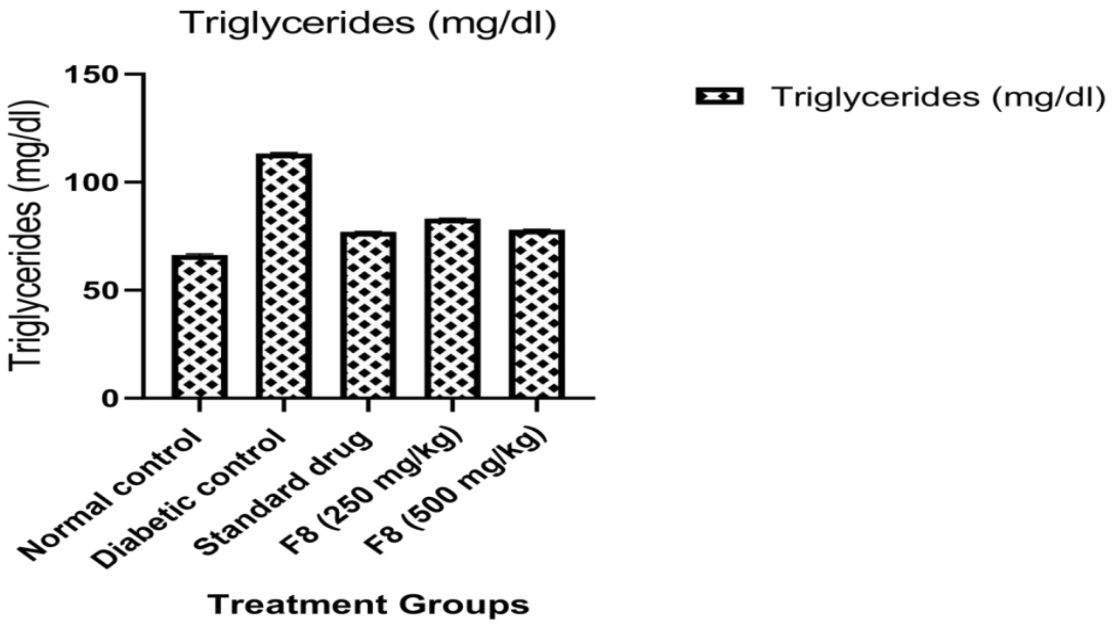

Figure 11: Effect on Triglycerides (mg/dl) level

Source: *Researcher's Data Analysis,2024*

The figure illustrates the effect of formulation F8 on serum triglyceride levels in STZ-induced diabetic rats. The diabetic control group showed significantly elevated triglyceride levels compared to the normal control group, indicating impaired lipid metabolism. Treatment with Glibenclamide significantly reduced serum triglyceride levels. Likewise, F8 film-coated tablets produced a dose-dependent reduction in triglycerides, with the 500 mg/kg dose showing greater hypolipidemic activity than the 250 mg/kg dose and approaching the effect of the standard treatment.

CONCLUSION:

The present investigation successfully developed and evaluated a polyherbal film-coated tablet formulation containing Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula, and Pavonia zeylanica for the management of diabetes mellitus and its associated metabolic complications. The optimized formulation (F8) demonstrated satisfactory pharmaceutical characteristics, indicating its suitability as a stable oral dosage form with acceptable quality attributes.

Pharmacological evaluation in streptozotocin-induced diabetic rats revealed that the formulation exhibited significant dose-dependent antidiabetic activity. Treatment with F8 markedly reduced fasting blood glucose and HbA1c levels while significantly improving serum insulin concentration and oral glucose tolerance. In addition, the formulation restored serum total protein levels, suggesting improvement in overall metabolic function and pancreatic β-cell activity.

The formulation also produced pronounced anti-inflammatory effects by significantly reducing the circulating levels of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β. Furthermore, the antihyperlipidemic study demonstrated a substantial improvement in lipid profile, evidenced by reductions in total cholesterol, LDL cholesterol, triglycerides, and VLDL levels, along with improvement in HDL cholesterol. These findings indicate that the formulation effectively addresses both hyperglycemia and diabetes-associated dyslipidemia, thereby reducing the risk of long-term cardiovascular complications.

The observed therapeutic efficacy may be attributed to the synergistic interaction of diverse phytoconstituents such as flavonoids, phenolic compounds, tannins, alkaloids, glycosides, and terpenoids present in the selected medicinal plants. These bioactive compounds collectively contribute to antioxidant, anti-inflammatory, insulin-sensitizing, and lipid-lowering activities through multiple complementary mechanisms.

Overall, the optimized polyherbal film-coated tablet demonstrated significant antidiabetic, anti-inflammatory, and antihyperlipidemic activities, with the higher dose (500 mg/kg) producing effects comparable to those of the standard drug. The findings support the potential of this polyherbal formulation as a safe and effective complementary therapeutic option for the management of diabetes mellitus and its associated complications. Nevertheless, further studies involving detailed toxicity evaluation, pharmacokinetic investigations, identification of active phytoconstituents, molecular mechanism studies, and well-designed clinical trials are warranted to confirm its long-term safety, efficacy, and therapeutic applicability in human subjects.

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