Research Article

Evaluation of Antidiabetic Potential of Gymnema Sylvestre and Metformin Combination in Streptozotocin-Induced Diabetic Rats

Ajay Kodiyatar[1], Niket Rai[1], Akhilesh Mishra[1], Vandana Roy[1]

1. Maulana Azad Medical College (MAMC), New Delhi, India

Background: Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder characterized by insulin resistance and hyperglycemia. Although metformin remains a first-line therapy, interest in plant-based adjuncts like Gymnemasylvestre (GS) is increasing due to their potential antidiabetic properties.

Objectives: The study aimed to assess the efficacy of the combination of Gymnemasylvestre (GS) and metformin in reducing blood glucose levels and body weight in streptozotocin-induced diabetic rats. Additionally, it sought to compare the effectiveness of this combination therapy with metformin alone in achieving glycemic control and weight reduction. The investigation also explored the potential benefits of the combination treatment on lipid profile and renal function, providing a broader understanding of its therapeutic impact in managing type 2 diabetes mellitus.

Method: Thirty male Sprague Dawley rats (150 ± 20 g) were divided into five groups: normal control, diabetic control, metformin-treated, GS-treated, and combination-treated. T2DM was induced by administering a high-fat diet for 21 days followed by two low-dose intraperitoneal injections of streptozotocin (25 mg/kg, five days apart). Rats with fasting blood glucose (FBG) ≥250 mg/dL were selected for treatment, which continued for 28 days.

Results: All treatment groups showed significant improvements in biochemical parameters compared to diabetic controls ($p < 0.05$). Metformin and combination therapy groups demonstrated greater reductions in FBG, cholesterol, creatinine, and HbA1c levels. While GS alone had modest antidiabetic effects, its combination with metformin enhanced efficacy, especially in glycemic control and lipid profile. Metformin alone showed superior effects on renal function.

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Conclusion: Gymnemasylvestre exhibits antidiabetic activity, which is amplified in combination with metformin. Although metformin alone remains more effective, the combination therapy offers additional benefits in T2DM management.

Correspondence: papers@team.qeios.com — Qeios will forward to the authors

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It poses a major global health concern due to its increasing prevalence and associated complications such as cardiovascular disease, neuropathy, and nephropathy.[1] Type 1 diabetes is an autoimmune condition involving the destruction of pancreatic β-cells, leading to absolute insulin deficiency. In contrast, type 2 diabetes mellitus (T2DM) is more prevalent and is characterized by insulin resistance and relative insulin deficiency.[2]

The management of T2DM commonly involves lifestyle modification and pharmacological therapy. Metformin, a biguanide and the first-line oral antidiabetic agent, enhances insulin sensitivity and reduces hepatic glucose production.[3] Despite its proven efficacy and safety, long-term use of metformin may be associated with gastrointestinal side effects and rare cases of lactic acidosis, necessitating the exploration of adjunct or alternative therapies.[4]

Gymnemasylvestre, a traditional Indian medicinal plant, has gained attention for its antidiabetic properties. Active constituents such as gymnemic acids have demonstrated hypoglycemic, anti-inflammatory, and antioxidant effects.[5] These compounds are thought to enhance insulin secretion, improve peripheral glucose uptake, and inhibit intestinal glucose absorption.[6]

Combining herbal therapies like G. sylvestre with standard drugs such as metformin may improve glycemic control and mitigate adverse effects associated with conventional therapy. Previous studies suggest that such combinations may offer synergistic benefits by targeting multiple pathophysiological mechanisms involved in T2DM.[7]

The present study aimed to evaluate the antidiabetic efficacy of G. sylvestre and metformin, individually and in combination, in a high-fat diet and streptozotocin-induced rat model of T2DM. This model

effectively mimics both insulin resistance and β-cell dysfunction, making it a suitable platform for assessing potential therapeutic interventions.[8]

Materials and Methods

Drugs, Chemicals, and Supplements

- Streptozotocin (STZ): Obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) in 500 mg vials[9].
- Gymnemasylvestre Leaf Extract: Procured from Arjuna Natural Pvt. Ltd. (Kerala, India), supplied in 100 g powder form[10].
- Metformin: Administered as OKAMET 500 mg tablets (Cipla Ltd., Haridwar, India).
- High-Fat Diet (HFD): Composed of 60% fat, 20% protein, and 20% carbohydrates, sourced from KaryomePvt. Ltd. (Mysuru, India).
- Other Reagents: Citric acid, sodium citrate, and sodium carboxymethyl cellulose (Na-CMC) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used to prepare the citrate buffer.

Experimental Animals

Thirty male Sprague Dawley rats (6-8 weeks old, 150 ± 20 g) were obtained from the Central Animal Facility, MAMC. Rats were housed three per cage under standard laboratory conditions (25 ± 5 °C temperature, 55 ± 10% humidity, 12-hour light/dark cycle). All animals had ad libitum access to food and water. A one-week acclimatization period was allowed before initiating experiments. Animals were randomly allocated into five groups using computer-generated randomization.

Experimental Design

Phase 1: Induction of Type 2 Diabetes Mellitus (T2DM)

T2DM was induced using the fat-fed/STZ model. Rats were fed a high-fat diet (HFD) for 21 days to induce insulin resistance. Body weight was monitored weekly during this period. On Days 22 and 27, rats received two intraperitoneal injections of STZ (25 mg/kg body weight) prepared in 0.1 mM citrate buffer (pH 4.5). Fasting blood glucose (FBG) was measured three days after the second injection using a tail vein

blood sample and glucometer. Rats with FBG ≥250 mg/dL were considered diabetic and enrolled for treatment.[8][9]

Phase 2: Drug Administration

Diabetic rats were randomly assigned to four groups (n = 6 per group), while non-diabetic rats were assigned to the normal control group:

- Group 1 (Normal Control): Normal rats received normal saline (10 mL/kg) via oral gavage for 4 weeks.
- Group 2 (Diabetic Control): Diabetic rats received normal saline (10 mL/kg) for 4 weeks.
- Group 3 (Metformin): Diabetic rats received metformin (200 mg/kg/day) via oral gavage for 4 weeks.[10]
- Group 4 (Gymnemasylvestre): Diabetic rats received G. sylvestre extract (600 mg/kg/day) via oral gavage for 4 weeks.[11]
- Group 5 (Metformin + G. sylvestre): Diabetic rats received both metformin (200 mg/kg/day) and G. sylvestre (600 mg/kg/day) via oral gavage for 4 weeks.

Dosages were calculated based on previously published studies and the human equivalent dose (HED) conversion method.[10]

Blood Sample Collection

Blood samples were collected via the retro-orbital plexus under ketamine (80 mg/kg) and xylazine (100 mg/kg) anesthesia. Approximately 0.4–0.5 mL of blood was collected into microcentrifuge tubes on Days 0, 7, and 28 for FBG analysis, and on Days 0 and 28 for HbA1c, serum creatinine, and total cholesterol. Samples were centrifuged at 4000 g for 15 minutes at 4 °C, and plasma was stored at −80 °C until analysis using a Semi-Automated Biochemistry Analyzer.

Statistical Analysis

All values were expressed as mean ± standard deviation (SD). Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant. Data analysis was conducted using SPSS software version 20.0 (Chicago, IL, USA).

Results

Diabetes Induction and Baseline Parameters

All rats began with comparable baseline body weights and fasting blood glucose (FBG) levels. After high-fat diet feeding and STZ administration, significant hyperglycemia was observed in all diabetic groups ($p < 0.05$ vs. normal control), confirming successful T2DM induction.

Group	Weight (gm)		FBG (mg/dl)
	Baseline	Pre-treatment (Day 0)	Pre-treatment (Day 0)
NC	163.16 ± 9.95	265.83 ± 21.77	101.16 ± 12.78
DC	163.16 ± 6.64	302.83 ± 56.02	476.00 ± 80.88*
Met	158.16 ± 5.38	235.50 ± 22.98	461.50 ± 13.50*
GS	154.66 ± 4.84	269.50 ± 32.77	448.66 ± 10.26*
Met+GS	156.00 ± 6.54	280.16 ± 24.01	436.33 ± 27.78*

Table 1. Induction of Diabetes (Pre-treatment Data)

**Values are mean ± SEM; n = 6 per group*

**Significant vs. NC ($p < 0.05$)*

Inference

No significant differences were observed in baseline body weights ($p > 0.05$). However, post-STZ injection, all experimental groups showed significantly elevated FBG levels compared to the normal control group, indicating successful T2DM induction.

Treatment Effects on Biochemical and Physiological Parameters

After 4 weeks of treatment, significant reductions in FBG, cholesterol, creatinine, and HbA1c were observed in the Met, GS, and Met + GS groups compared to the diabetic control (DC) group ($p < 0.05$). Metformin alone showed the most pronounced effect.

Group	Weight (gm)	FBG (mg/dl)		Cholesterol (g/L)		Creatinine (mg/dl)		Hb1Ac (g/L)	
	Day 28	Day 7	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
DC	293.0 ±	445.66 ±	401.33 ±	351.6 ±	345.14±	2.05 ±	1.85±	10.55 ±	9.95 ±
	33.41	51.74	24.548	75.99	70.77	0.47	0.04	0.57	0.49
Met	389.8 ±	409.66 ±	122.50 ±	331.6 ±	106.49 ±	1.85 ±	0.44 ±	10.65 ±	5.82 ±
	19.53*	8.16*	6.15*	10.06	2.73*	0.76	0.03*	0.11	0.15*
GS	393.0 ±	423.83 ±	296.33 ±	323.9 ±	293.43 ±	1.77 ±	0.69 ±	10.62 ±	8.24 ±
	30.21*	13.61	8.26*†	16.91	3.29*†	0.89	0.04*†	0.27	0.13*†
Met+	421.8 ±	395.83 ±	176.50 ±	315.7 ±	208.99 ±	1.74 ±	0.54 ±	10.65 ±	7.26 ±
GS	27.96*†	10.88*†	9.20*†	14.53	1.18†	0.12	0.02*†	0.70	0.09*†

Table 2. Effects of Treatments on Day 28

**Significant vs. DC ($p < 0.05$)*

†Significant vs. Met ($p < 0.05$)

Inference

- All treatment groups showed improvements vs. DC.
- Metformin was most effective in reducing FBG, cholesterol, creatinine, and HbA1c.
- The Met + GS combination enhanced effects over GS alone but was slightly less effective than Met alone.

Fasting Blood Glucose Over Time

FBG levels were significantly reduced by Day 7 and Day 28 in Met and Met + GS groups compared to DC. GS alone showed modest improvement by Day 28 but was not significant by Day 7.

Statistical Highlights

- Day 7: Significant FBG reduction in Met ($p = 0.030$) and Met + GS ($p = 0.004$) vs. DC.

- Day 28: All treatment groups showed significant reduction ($p < 0.001$).
- Met outperformed GS and Met + GS in terms of magnitude of FBG reduction.

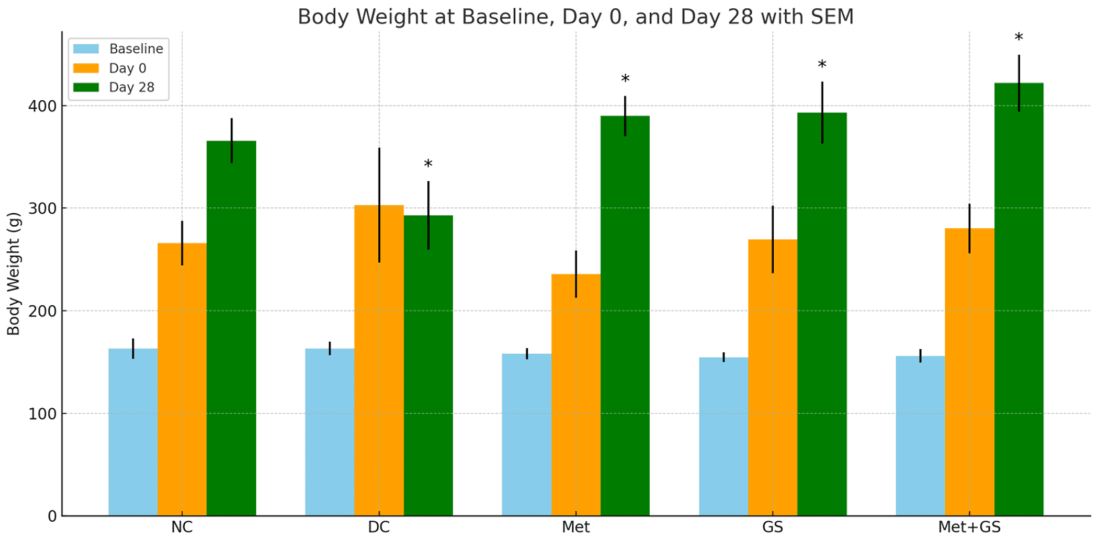

Figure 1. Body Weight at Baseline, Day 0, and Day 28

- No significant differences at baseline and Day 0.
- Day 28: Significant weight gains in Met, GS, and Met + GS compared to DC.

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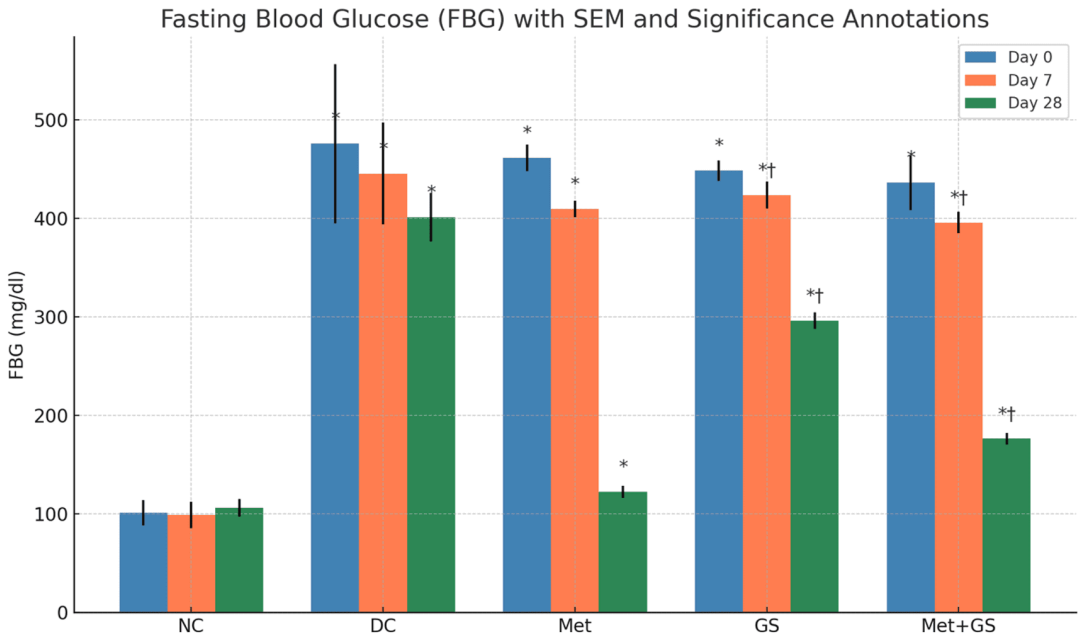

Figure 2. FBG at Day 0, Day 7, and Day 28

- Met and Met + GS showed rapid and sustained reductions.
- GS group exhibited gradual reduction by Day 28.

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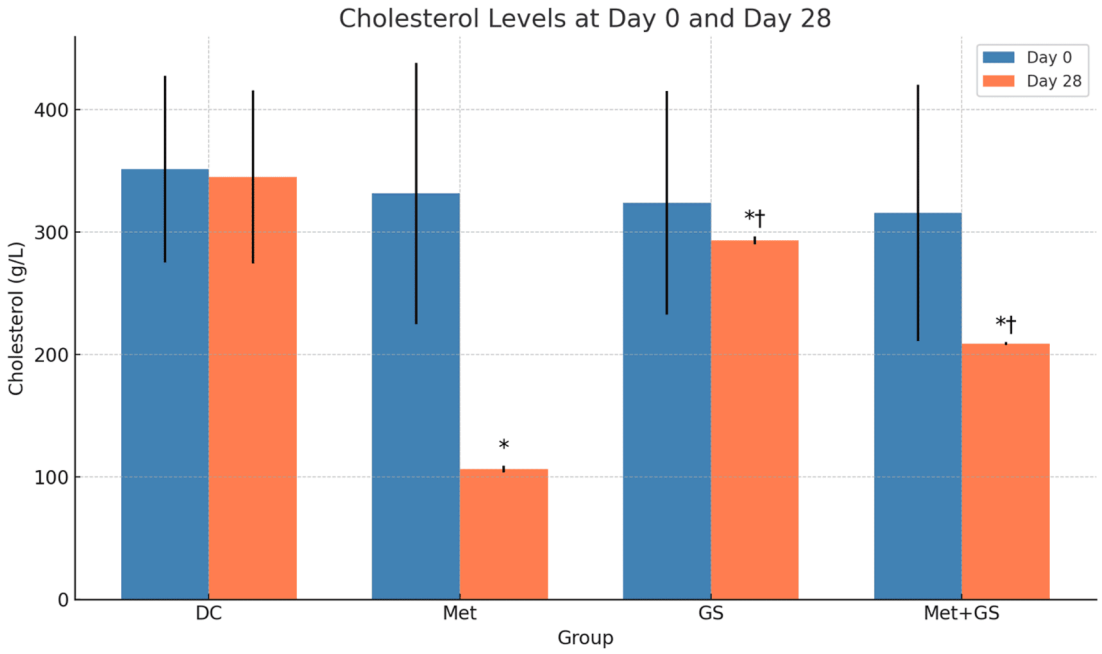

Figure 3. Cholesterol at Day 0 and Day 28

- All treatments significantly reduced cholesterol; Met showed the greatest effect.

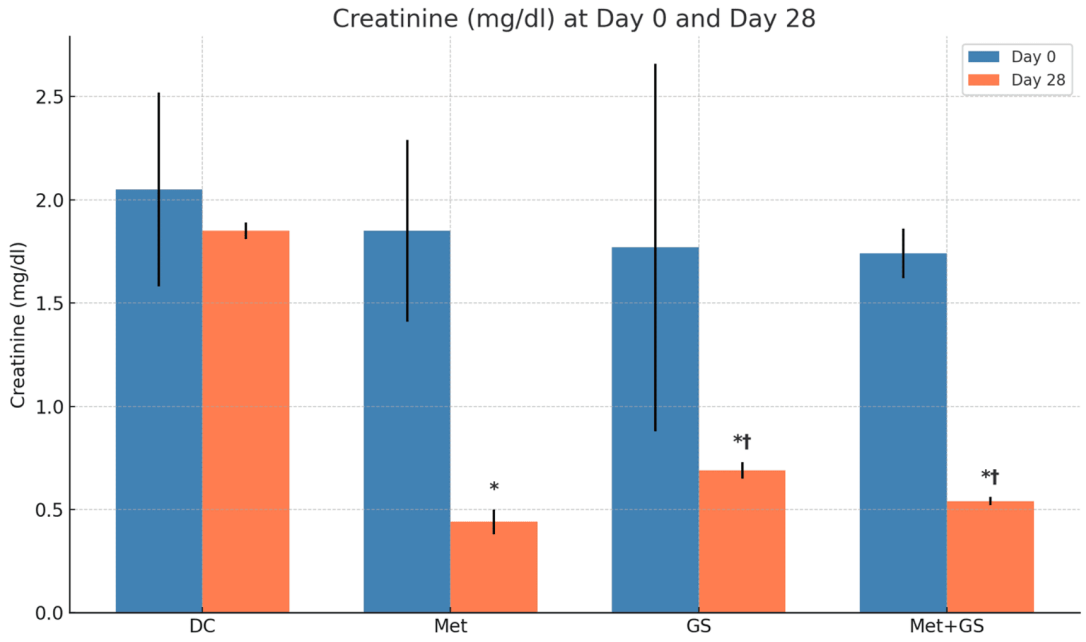

Figure 4. Creatinine at Day 0 and Day 28

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- All treatments improved renal function, with Met being the most effective.

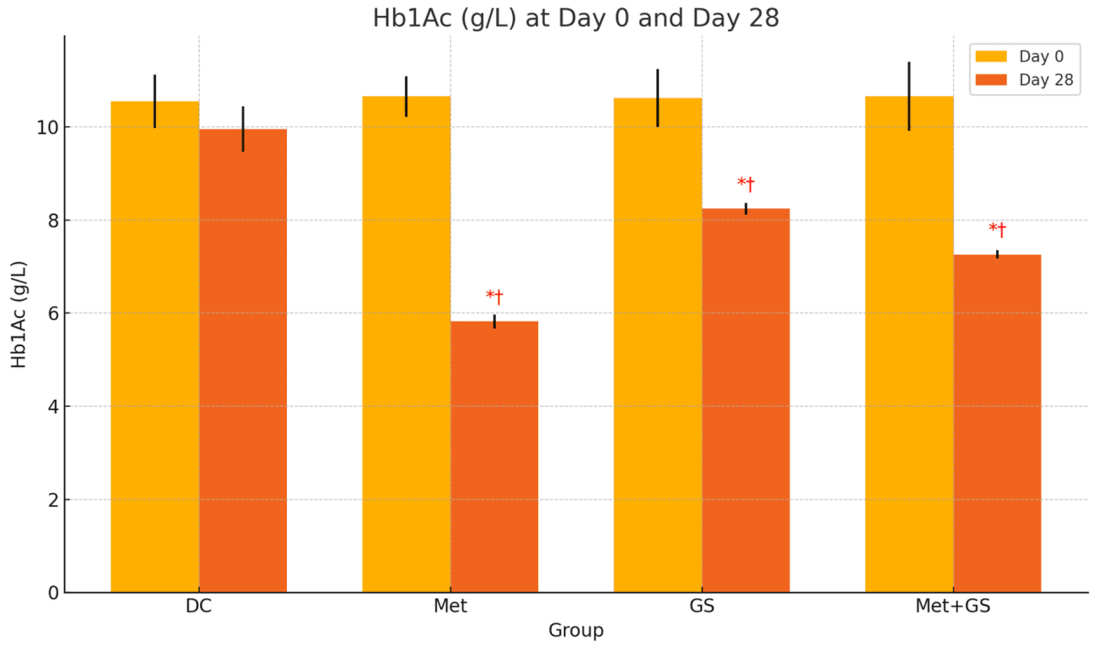

Figure 5. HbA1c at Day 0 and Day 28

- Met and Met + GS significantly reduced HbA1c levels, followed by GS alone.

Discussion

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder marked by chronic hyperglycemia due to insulin resistance and impaired insulin secretion. Its global prevalence is rapidly increasing, posing a significant public health challenge. While current therapies like metformin are effective, there remains a need for improved treatment strategies with enhanced efficacy and fewer side effects.[1][3]

In this study, T2DM was successfully induced in rats using a combination of a high-fat diet (HFD) and streptozotocin (STZ), simulating the dual pathophysiological defects observed in human T2DM, namely, insulin resistance and pancreatic β-cell dysfunction.[8][9] This model remains one of the most reliable for evaluating the efficacy of potential antidiabetic agents.

Metformin, used as the standard treatment in this study, significantly improved glycemic control and metabolic parameters, including fasting blood glucose (FBG), total cholesterol, serum creatinine, and

HbA1c. These findings align with established evidence showing metformin's ability to enhance insulin sensitivity, reduce hepatic gluconeogenesis, and improve lipid and renal profiles.[3][12]

Gymnemasylvestre, a medicinal plant with a long history in Ayurvedic practice, also showed a glucose-lowering effect, though it was more modest compared to metformin. This effect is likely mediated through gymnemic acids, which are known to reduce intestinal glucose absorption, enhance insulin secretion, and protect pancreatic β-cells from oxidative stress.[4][13][14]

Interestingly, the combination therapy of G. sylvestre with metformin demonstrated enhanced improvements over G. sylvestre alone in all measured parameters. However, the combination did not outperform metformin monotherapy in most outcomes. This suggests a possible additive, but not synergistic, interaction between the two agents. These findings are consistent with prior reports that herbal-drug combinations may provide benefit by targeting multiple metabolic pathways.[8][15]

The observed reductions in cholesterol and creatinine levels indicate beneficial effects beyond glycemic control, particularly on lipid metabolism and renal function. Given that diabetic nephropathy and dyslipidemia are common complications of T2DM, such effects are clinically relevant.[12][16]

Nevertheless, while G. sylvestre contributes to glycemic regulation, its relatively weaker effect compared to metformin underlines the importance of standard pharmacological agents. However, its role as an adjunct may be valuable, especially for patients who are intolerant to high doses of metformin or who prefer integrative therapeutic approaches.

This study demonstrated that both metformin and Gymnemasylvestre, individually and in combination, exert significant antidiabetic effects in STZ-induced diabetic rats. Metformin was the most effective agent, significantly reducing fasting blood glucose, total serum cholesterol, serum creatinine, and HbA1c levels. G. sylvestre showed moderate efficacy, and its combination with metformin provided additional, although not synergistic, benefits.

Limitation

Despite the effective establishment of a high-fat diet and low-dose streptozotocin-induced rat model of type 2 diabetes, this work has significant drawbacks. The 28-day treatment period may not be sufficient to evaluate long-term effectiveness, safety, or toxicity. The sample size is modest (n = 6 for each group), which may restrict statistical power and generalizability. Functional evaluations such as oral glucose tolerance tests (OGTT) and insulin tolerance tests (ITT) were not conducted, which might have provided

further information about glucose metabolism. Furthermore, the study did not investigate the biological processes driving insulin resistance, such as insulin receptor signalling or GLUT4 expression. Histopathological examinations of the pancreas, liver, and kidneys were also excluded, restricting the morphological confirmation of biochemical results. Complications such as neuropathy and nephropathy were not examined. While the animal model resembles many elements of human diabetes, it does not convey the disease's complete complexity. Clinical investigations are required to confirm these findings in human populations.

Conclusion

This study demonstrated that both metformin and Gymnemasylvestre, individually and in combination, exert significant antidiabetic effects in STZ-induced diabetic rats. Metformin was the most effective agent, significantly reducing fasting blood glucose, total serum cholesterol, serum creatinine, and HbA1c levels. G. sylvestre showed moderate efficacy, and its combination with metformin provided additional, although not synergistic, benefits.

These findings suggest that G. sylvestre may serve as a supportive adjunct to metformin in the management of type 2 diabetes mellitus. However, metformin remains the more potent and reliable agent. Further studies involving long-term administration, molecular mechanisms, and clinical validation are recommended to confirm these results and evaluate safety and efficacy in humans.

Statements and Declarations

Study Setting and Ethical Approval

This study was conducted at the Central Animal Facility and Department of Pharmacology, Maulana Azad Medical College (MAMC), New Delhi. Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) under approval numberIAEC/MAMC/CAF/2023/03, dated 28-04-2023.

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Declarations

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