Research Article

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

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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 *Gymnema sylvestre* is increasing due to their potential antidiabetic properties.

Objectives: The study aimed to assess the efficacy of the combination of G. sylvestre 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) ≥270 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.

Conclusion: G. sylvestre exhibits antidiabetic activity, which is amplified in combination with metformin. Although metformin alone remains more effective, the combination therapy offers

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additional benefits in T2DM management.

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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 I diabetes, an autoimmune illness, is primarily characterized by the death of pancreatic beta cells, resulting in inadequate insulin production. Insulin therapy is used to manage blood glucose levels and avoid problems. Type II diabetes is distinguished by insulin resistance and reduced insulin secretion. The primary therapy for type II diabetes is the use of oral antidiabetic medications like metformin, which increases insulin sensitivity. It works largely by lowering hepatic glucose production, increasing peripheral glucose absorption, and improving insulin sensitivity.[2] Metformin has been found to help manage hyperglycemia and reduce diabetic complications. However, long-term treatment may result in gastrointestinal side effects and lactic acidosis, emphasizing the importance of finding alternative medicines, particularly those that reduce side effects and improve therapeutic results.[3]

Gymnema sylvestre (G. sylvestre) Also known as Gurmar (Hindi), G. sylvestre is a woody climber in the Asclepiadaceae family, often referred to as the "milkweed" family. G. sylvestre has been used for over 2000 years in Indian Ayurvedic medicine to treat diabetes.[4] The major chemical ingredient, gymnemic acid, has anti-inflammatory, anti-sugary, and antidiabetic properties. These compounds are thought to enhance insulin secretion, improve peripheral glucose uptake, and inhibit intestinal glucose absorption.[5]

Streptozotocin (STZ) is a strong substance that preferentially damages pancreatic beta-cells, resulting in insulin insufficiency and hyperglycemia, making it a popular chemical for inducing diabetes in laboratory animals.[6] The STZ-induced diabetes rat model is widely used to assess possible antidiabetic treatments. In this regard, the study of the combined antidiabetic potential of G. sylvestre and metformin in STZ-induced diabetic rats may give useful insights into the advantages of such a combination for diabetes therapy.

Despite this, the combination of G. sylvestre and metformin has not been well investigated in known diabetic animal models. There is a significant research gap on whether this combination provides additive or synergistic effects.

The current study aimed to assess the antidiabetic potential of a G. sylvestre and metformin combination in a streptozotocin-induced rat model of T2DM, with a focus on glycemic control, lipid profile, body weight, and renal function parameters.

Materials and Methods

Drugs, Chemicals, and Supplements

- Streptozotocin (STZ): Obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) in 500 mg vials.
- G. sylvestre leaf Extract (100 g powder) sourced from Arjuna Natural Pvt. Ltd. in Kerala, India. Preparation of Plant Extract: Hydroalcoholic extraction was performed on the powdered leaves of G. sylvestre.
- 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 Karyome Pvt. 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 ≥270 mg/dL were considered diabetic and enrolled for treatment.[6]

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.[7]
- Group 4 (*Gymnema sylvestre*): Diabetic rats received G. sylvestre (600 mg/kg/day) via oral gavage for 4 weeks.[8]
- 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.[7][8]

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 rpm 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). Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess differences between multiple treatment groups across time points. This method was chosen due to its robustness in detecting group differences in repeated measures and factorial designs. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 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)*

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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.

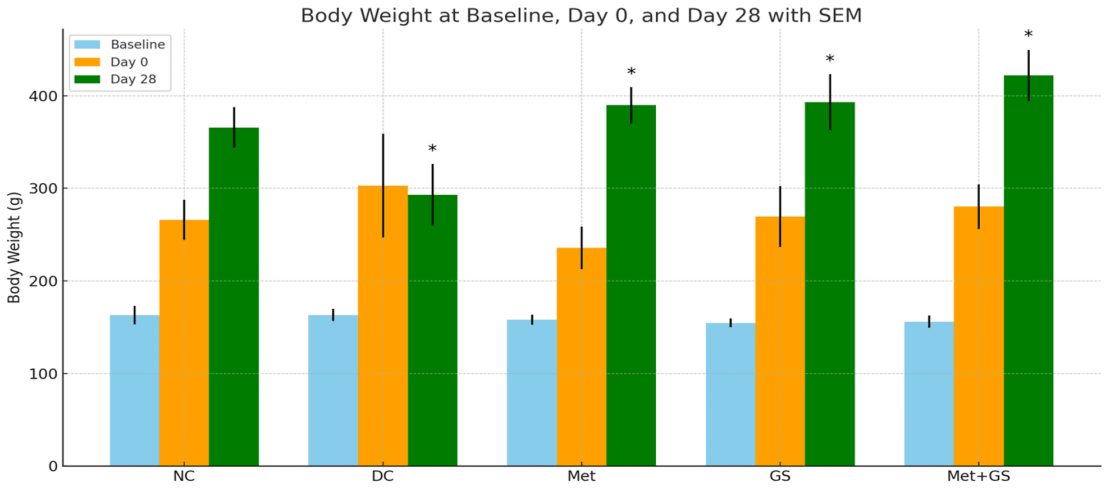

Figure 1. Body Weight at Baseline, Day 0, and Day 28

All comparisons show p-values < 0.001, indicating statistically significant weight differences between DC and the other groups on Day 28.

The normal control group, which received a normal pellet diet, had significantly lower weights compared to the other groups, which received HFD/STZ.

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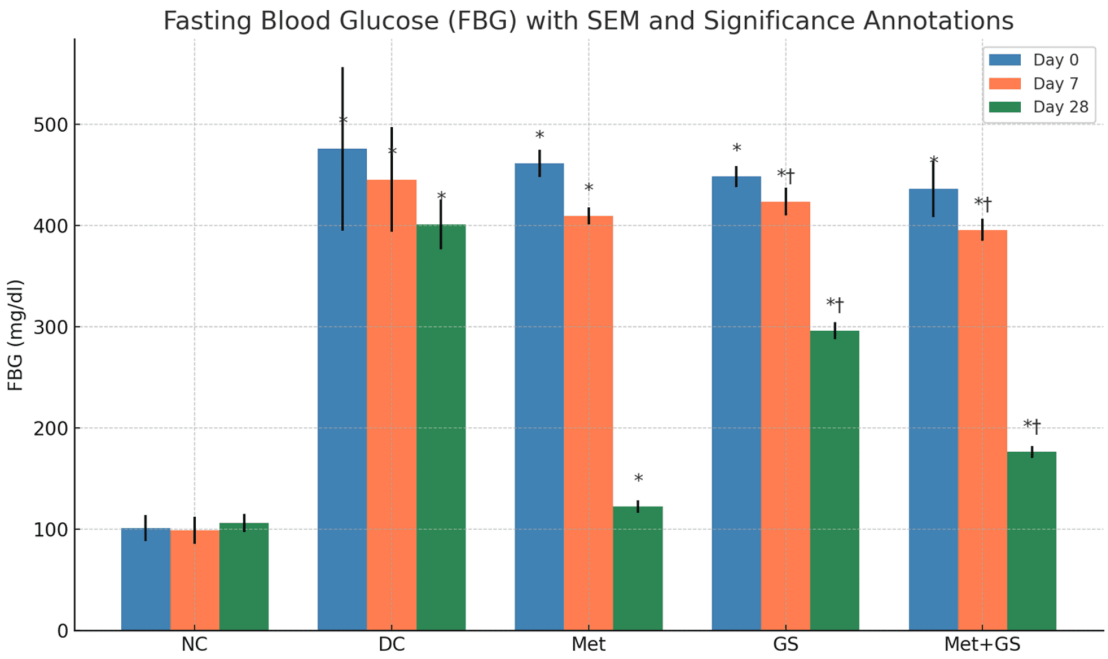

Figure 2. Fasting Blood Glucose (FBG) with SEM and Significance Annotations

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.

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

All comparisons exhibited p-values < 0.001, indicating statistically significant differences (reductions) in fasting blood glucose levels between DC and the other groups on Day 28. Using two-way RM ANOVA, inter-group comparisons at various time periods revealed that the Met, GS, and Met+GS groups had substantially lower levels of fasting blood glucose at the end of the study compared to the DC group.

The statistical analysis found significant differences between the Met and Met+GS groups with p-values of 0.01 ($p<0.05$) and between the Met and GS groups. (Table 2; Figure 2)

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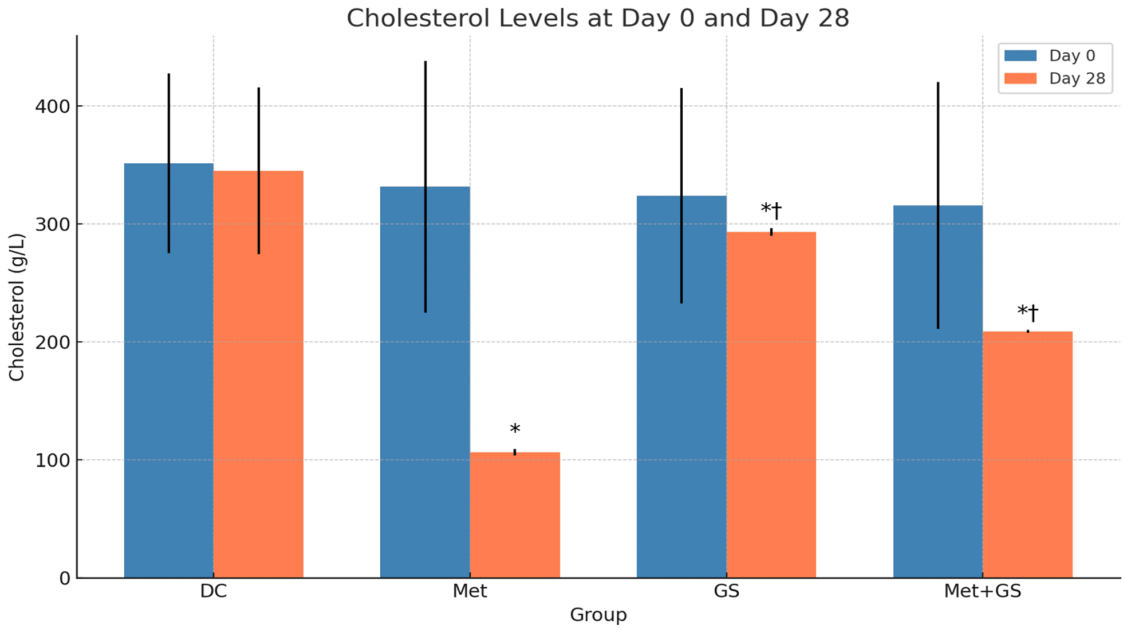

Figure 3. Cholesterol Levels at Day 0 and Day 28

All comparisons revealed p-values < 0.001, indicating statistically significant differences (reductions) in total serum cholesterol between DC and the other groups on Day 28. Using two-way RM ANOVA, inter-group comparisons at various time periods revealed that the Met, GS, and Met+GS groups had substantially lower total blood cholesterol levels at the end of the study compared to the DC group.

The statistical analysis found significant differences between the Met and Met+GS groups with p-values of 0.01 ($p<0.05$) and between the Met and GS groups (Table 2, Fig. 3).

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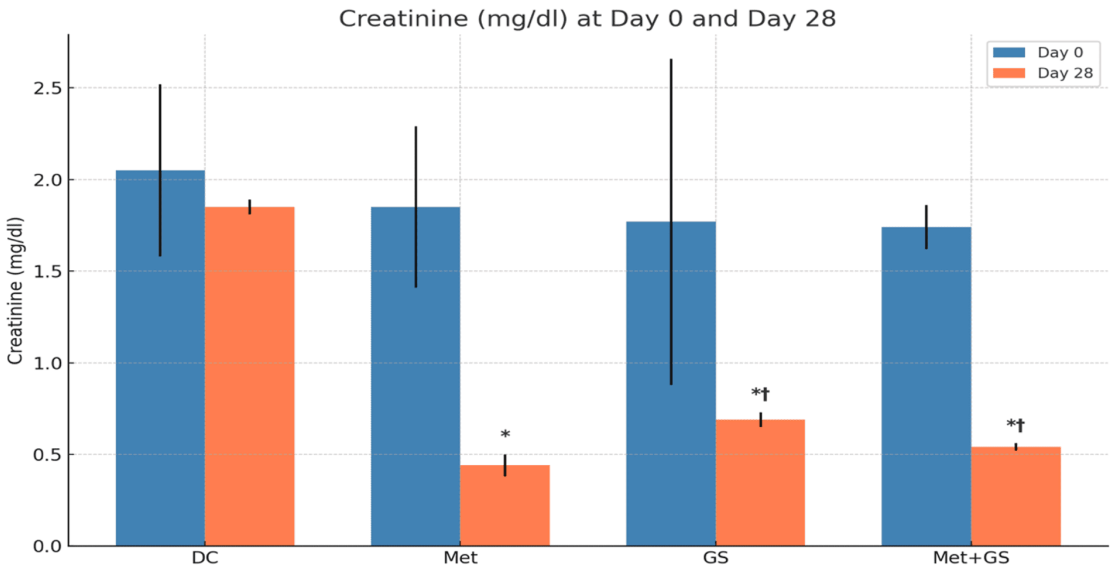

Figure 4. Creatinine (mg/dl) at Day 0 and Day 28

All comparisons exhibited p-values < 0.001, indicating statistically significant differences (reductions) in blood creatinine levels between DC and the other groups on Day 28. Using two-way RM ANOVA, inter-group comparisons at various time periods revealed that the Met, GS, and Met+GS groups had substantially lower blood creatinine levels at the end of the study compared to the DC group.

The statistical analysis found significant differences between the Met and Met+GS groups with p-values of 0.01 ($p<0.05$) and between the Met and GS groups. (Table 2; Figure 4).

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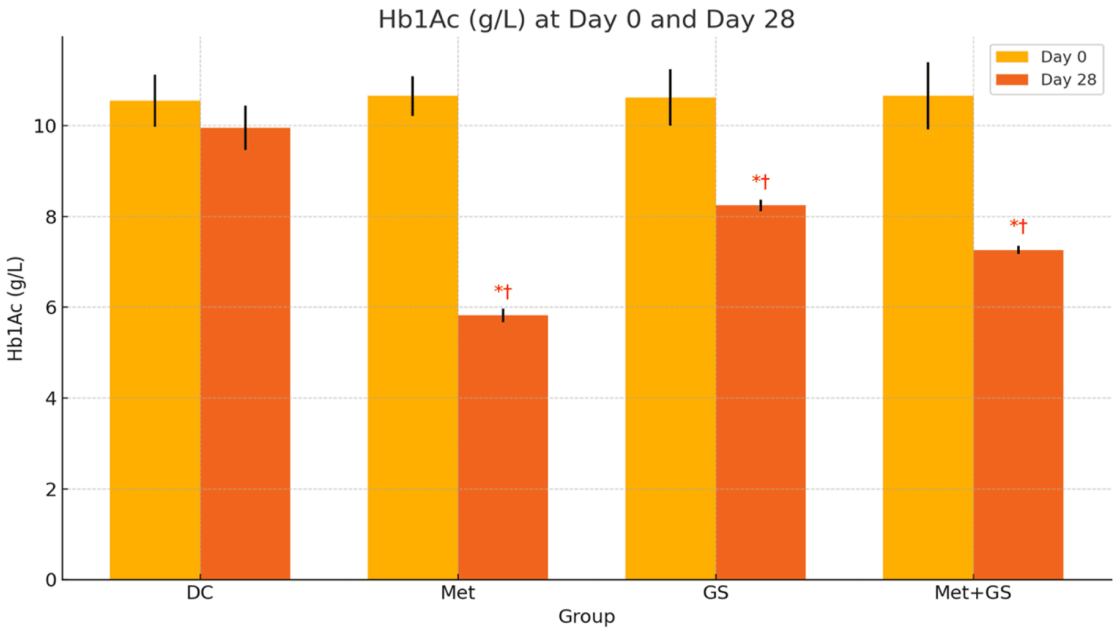

Figure 5. Hb1Ac (g/L) at Day 0 and Day 28

All comparisons exhibited p-values < 0.001, indicating statistically significant differences (reductions) in HbA1c between DC and the other groups on Day 28. Using two-way RM ANOVA, inter-group comparisons at various time periods revealed that the Met, GS, and Met+GS groups had substantially lower HbA1c values at the end of the study compared to the DC group.

The statistical analysis found significant differences between the Met and Met+GS groups with p-values of 0.01 ($p<0.05$) and between the Met and GS groups. (Table 2; Figure 5)

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.[9]

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.[6] 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.[10] Moreover, the nephroprotective effects observed may be attributed to the activation of the AMPK pathway, a mechanism supported by recent research highlighting metformin's protective role against diabetic complications.[10]

G. sylvestre, a medicinal plant with a long history in Ayurvedic practice, also demonstrated glucose-lowering effects, although more modest compared to metformin. This effect is likely mediated through its active constituents, gymnemic acids, which have been shown to reduce intestinal glucose absorption, stimulate insulin secretion, and protect pancreatic β-cells from oxidative damage.[11][12]

Interestingly, the combination therapy of G. sylvestre with metformin showed enhanced improvements over G. sylvestre alone in all measured parameters. However, the combination did not significantly outperform metformin monotherapy in most outcomes. This suggests a possible additive rather than synergistic interaction between the two agents. Such findings are consistent with prior studies showing that herbal-drug combinations may provide complementary benefits by targeting multiple metabolic pathways, including insulin sensitivity, oxidative stress, and β-cell function.[6][13]

The observed reductions in cholesterol and creatinine levels indicate that the combination therapy may also confer protective effects on lipid metabolism and renal function. These improvements are particularly relevant, given the high prevalence of diabetic dyslipidaemia and nephropathy in T2DM patients.[10][14]

Nonetheless, while G. sylvestre exhibits potential as an adjunct therapy, its relatively lower efficacy highlights the importance of conventional agents like metformin as the cornerstone of T2DM management. The adjunct use of G. sylvestre may be especially valuable in patients seeking integrative approaches or those intolerant to higher doses of metformin.

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

Limitations and Future Directions

This study, while offering valuable insights into the antidiabetic potential of *Gymnema sylvestre* and metformin combination therapy, has several limitations. The 28-day treatment duration may not adequately reflect long-term safety or therapeutic efficacy. A small sample size (n = 6 per group) limits the statistical power and generalizability of the findings. Additionally, the absence of functional assessments such as oral glucose tolerance tests (OGTT) and insulin tolerance tests (ITT) restricts understanding of the treatment's effect on glucose metabolism and insulin sensitivity. Histological analysis of key organs such as the pancreas, liver, and kidneys was not performed, thereby limiting the correlation between biochemical changes and structural alterations. The lack of phytochemical profiling also leaves the identity and contribution of the active compounds in G. sylvestre undefined. To address these limitations, future studies should incorporate larger sample sizes, extended treatment periods, and comprehensive functional tests. Advanced techniques like LC-MS/MS for compound identification and molecular docking to predict compound–target interactions should be employed to explore the underlying mechanisms of action. Investigating insulin signalling pathways, particularly those involving GLUT4 and IRS-1, could provide mechanistic depth. Finally, clinical trials are essential to validate these preclinical results and establish the translational potential of this combination therapy in human populations.

Conclusion

This study demonstrated that both metformin and G. sylvestre, 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 number IAEC/MAMC/CAF/2023/03, dated 28-04-2023.

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Declarations

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Potential competing interests: No potential competing interests to declare.