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# In-Vitro and In-Vivo Evaluation of the Antidiabetic Potential and Antioxidant Properties of Selected GS Extract in DIO Mice Model

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**Abstract:**
Diabetes mellitus (DM) is a persistent metabolic disorder marked by elevated blood glucose levels (hyperglycemia), arising from impaired insulin secretion, defective insulin action, or a combination of both. It is one of the most prevalent non-communicable diseases globally and is considered a major public health challenge of the 21st century. This study was undertaken to evaluate the antidiabetic and antioxidant potential of the methanolic extract of *Gymnema sylvestre* through both in-vitro and in-vivo approaches, with particular emphasis on the DIO mouse model. The findings clearly demonstrate that the extract exhibits significant hypoglycemic, hypolipidemic, and antioxidant activities, thereby supporting its ethnopharmacological relevance in the management of diabetes mellitus. Overall, the *Gymnema sylvestre* extract appears to work through multiple pathways it slows sugar absorption, enhances antioxidant defenses, and protects key organs. Its effectiveness was comparable to the diabetes drug metformin, making it a promising natural option for managing diabetes.

**Keywords:** Diabetes mellitus, hyperglycemia, DIO Mice Model, GS Extract, Antioxidant

## Introduction

**Plant Profile: *Gymnema sylvestre***

**Botanical Description**

*Gymnema sylvestre* (family: Apocynaceae; synonym: *Asclepiadaceae*), commonly known as "Gurmar" or "sugar destroyer," is a perennial woody climber native to central and southern India, Sri Lanka, and parts of Africa. The plant grows in tropical forests, characterized by:

- Leaves: Opposite, elliptic, or ovate with soft hairs on the lower surface.
- Flowers: Small, yellowish, arranged in umbellate cymes.
- Fruits: Follicles containing flat seeds.

Its Hindi name *"Gurmar"* literally translates to "sugar destroyer," reflecting its long-standing use in traditional medicine for diabetes management.

***Fig.1:** Gymnema sylvestr*

**Traditional and Ethnomedicinal Uses**

*G. sylvestre* has been extensively used in Ayurvedic medicine for over 2,000 years. Traditional applications include:

- Antidiabetic: Chewing fresh leaves reduces the ability to perceive sweet taste.
- Digestive disorders: Used for constipation, indigestion, and gastric complaints.
- Other uses: Treatment of asthma, arthritis, obesity, snakebite, and cardiovascular diseases.

**Phytochemical Constituents**

Phytochemical studies have revealed that *G. sylvestre* is rich in bioactive constituents, particularly triterpene saponins collectively known as gymnemic acids, which are primarily responsible for its antidiabetic activity. Other reported compounds include:

- Gymnemic acids I–XX: Inhibit intestinal glucose absorption and suppress sweet taste sensation.
- Gymnemasaponins & gymnemagenin: Contribute to hypoglycemic effect.
- Flavonoids and phenolic compounds: Provide antioxidant and free-radical scavenging activity.
- Alkaloids, tannins, and anthraquinones: Supporting roles in metabolic regulation.

**Pharmacological Activities**

1. **Antidiabetic activity**
    - ***In vitro*:** Gymnemic acids inhibit intestinal enzymes (α-glucosidase, α-amylase), reducing carbohydrate digestion and absorption.
    - ***In vivo*:** Animal studies show improved glucose tolerance, increased insulin secretion, and β-cell regeneration.

- *Clinical studies*: Supplementation with *G. sylvestre* leaf extract reduces FBG and HbA1c levels in T2DM patients.

2. **Antioxidant properties**
   - Methanolic extracts exhibit strong scavenging activity in DPPH, ABTS, and FRAP assays.
   - Protects β-cells from oxidative injury and improves antioxidant enzyme activities (SOD, CAT, GPx).
3. **Lipid-lowering effects**
   - Reduces serum cholesterol, triglycerides, and LDL, while elevating HDL levels in hyperlipidemic models.
4. **Other reported effects**
   - Antimicrobial, anti-inflammatory, Anti-obesity, hepatoprotective activities.

**Relevance to the Present Study**

The dual antidiabetic and antioxidant properties of *Gymnema sylvestre* make it a promising candidate for diabetes management. Its bioactive saponins not only reduce glucose absorption and enhance insulin secretion but also mitigate oxidative stress, thereby addressing both glycemic control and complication prevention. Given its traditional reputation, proven phytochemical richness, and modern pharmacological evidence, *G. sylvestre* is an ideal plant for systematic evaluation in a diet-induced obesity (DIO) mouse model.

## Materials and Methods

**Plant Material**

Fresh leaves of Gymnema sylvestre were collected from the forest region of Karimnagar during the optimal harvesting season to ensure phytochemical integrity. The plant specimen was authenticated by a qualified taxonomist, and a voucher specimen (Herbarium No. 124/2024) was deposited for future reference. Following collection, the leaves were thoroughly rinsed with distilled water to eliminate surface impurities and debris. The cleaned material was shade-dried at ambient room temperature to preserve thermolabile constituents, then pulverized into a fine powder for subsequent extraction and analysis.

Chemicals: All solvents and reagents used in this study were of analytical grade. Methanol, ethanol, chloroform, and petroleum ether were purchased from Merck and Sigma-Aldrich. Biochemical assay kits for measuring glucose, triglycerides, total cholesterol, HDL, LDL, and HbA1c were obtained from certified commercial suppliers to ensure accurate and reproducible results. For enzyme inhibition studies, substrates such as soluble starch and p-nitrophenyl-α-D-glucopyranoside were utilized. Antioxidant assays incorporated key reagents including DPPH, ABTS, TPTZ (for the FRAP assay), sodium nitroprusside, and Griess reagent. Streptozotocin and alloxan were available for use in comparative antidiabetic evaluations. Metformin served as the standard reference drug for benchmarking antidiabetic efficacy. Male Swiss albino mice (25–30 g) or C57BL/6 mice were employed for the present study. Animals were obtained from the Central Animal Facility of [Institution Name] and housed under standardized laboratory conditions, including a controlled temperature of 22 ± 2 °C and a 12-hour light/dark cycle. Standard pellet diet and water were provided ad libitum throughout the experimental period. All animal-related experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The experiments were carried out following the ethical guidelines set by the CCSEA, Government of India.

**Methods**

**Preparation of GS extract**

After collection, the plant material was first thoroughly cleaned to remove dirt and foreign matter. To protect heat-sensitive compounds, it was then shade-dried at room temperature for 10-15 days. Once completely dry, the material was ground into a coarse powder and sieved to ensure a uniform particle size, which helps with efficient extraction. For the extraction of phytochemicals, a Soxhlet apparatus was used with analytical-grade methanol as the solvent. Methanol was chosen for its high polarity and ability to dissolve a wide range of secondary metabolites, such as alkaloids, flavonoids, glycosides, phenolic compounds, and terpenoids. Approximately 50–100 g of the powdered plant material was packed into a cellulose thimble and placed in the extraction chamber. The extraction ran continuously for 48–72 hours, or until the solvent was colorless, which indicated that all methanol-soluble components had been extracted. The resulting extract was then concentrated under reduced pressure using a rotary evaporator at 40°C. This controlled process efficiently removed the solvent while preserving heat-sensitive bioactive compounds.The concentrated extract was further dried in a desiccator to remove any remaining solvent, resulting in a semi-solid to solid mass. Finally, the dried extract was weighed, and the percentage yield was calculated using the formula below:

$$\text{Percentage yield (\% w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder taken}} \times 100$$

The containers were labelled and stored at 4 °C in a refrigerator until further use in phytochemical screening, pharmacological experiments.

**Phytochemical Analysis**

Preliminary phytochemical screening was carried out to identify the presence of major classes of bioactive constituents. Alkaloids were tested using Dragendorff's and Mayer's reagents, flavonoids were confirmed by the Shinoda test, phenolic compounds and tannins were detected using ferric chloride, saponins were identified by the frothing test, and terpenoids were determined using the Liebermann Burchard test. Quantitative estimation of phytochemicals included determination of total phenolic content by the Folin Ciocalteu method, flavonoid content by the aluminum chloride colorimetric method, and saponin content by gravimetric analysis.

**In-Vitro Antidiabetic Assays:** To determine the GS extract's antidiabetic properties, we investigated its ability to inhibit α-amylase and α-glucosidase, two key enzymes that break down carbohydrates. Enzyme inhibition assays were performed using standard spectrophotometric methods. These assays helped us quantify the extract's potential to manage blood sugar levels after meals by suppressing the activity of these enzymes. Since these enzymes play a crucial role in the digestion and absorption of dietary carbohydrates, inhibiting them is a common therapeutic approach for reducing post-meal hyperglycemia.

## Result and Discussion

The methanolic extract of *Gymnema sylvestre* was subjected to qualitative phytochemical screening, which confirmed the presence of key secondary metabolites such as alkaloids, flavonoids, phenolic compounds, saponins, and terpenoids. Tannins were detected in trace quantities, indicating their minimal contribution to the extract's overall phytochemical profile. Quantitative estimation showed that the total phenolic content was 65.2 ± 2.4 mg GAE/g extract, the total flavonoid content was 42.6 ± 1.8 mg QE/g extract, and saponins constituted 18.7 ± 0.9%. These findings confirm the richness of the extract in bioactive compounds known for their antidiabetic and antioxidant activities.

**Table.1:** Phytochemical Composition of *Gymnema* ***sylvestre*** Extract

| Phytochemical Test | Result | Quantitative Value |
|---|---|---|
| Alkaloids | Present | – |
| Flavonoids | Present | 42.6 ± 1.8 mg QE/g |
| Phenolics | Present | 65.2 ± 2.4 mg GAE/g |
| Saponins | Present | 18.7 ± 0.9 % |
| Terpenoids | Present | – |
| Tannins | Trace | – |

### *In-Vitro* Antidiabetic Activity

The methanolic extract of Gymnema sylvestre exhibited significant inhibitory effects on carbohydrate-hydrolyzing enzymes in a dose-dependent manner. The $IC_{50}$ value against α-glucosidase (98.6 ± 2.9 µg/mL) was lower than that for α-amylase (112.3 ± 3.6 µg/mL), suggesting stronger inhibition of α-glucosidase activity. Although the extract was less potent compared to acarbose (72.1 ± 2.4 µg/mL for α-glucosidase and 87.5 ± 2.8µg/mL for α-amylase), the results demonstrate that the GS extract can modulate postprandial hyperglycemia by delaying carbohydrate digestion and absorption.

Table 2: Enzyme Inhibitory Activity of *Gymnema sylvestre* Extract

| Assay | $IC_{50}$ Value (µg/mL) | Standard Drug (Acarbose) $IC_{50}$ (µg/mL) |
|---|---|---|
| α-Amylase Inhibition | 112.3 ± 3.6 | 87.5 ± 2.8 |
| α-Glucosidase Inhibition | 98.6 ± 2.9 | 72.1 ± 2.4 |

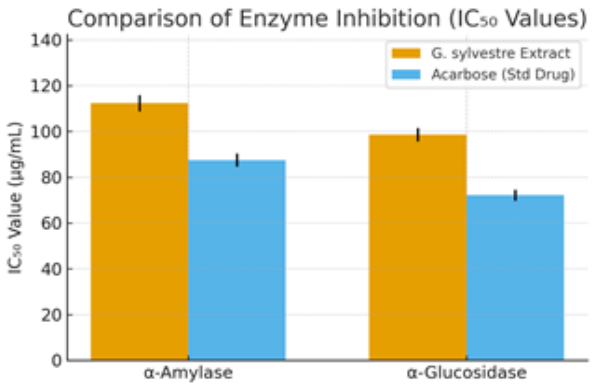


**Fig.2:** Comparison of Enzyme inhibition

### *In Vitro* Antioxidant Assays

The extract exhibited strong antioxidant activity across different assay systems. The $IC_{50}$ values for DPPH, ABTS scavenging were 76.2 and 69.5 µg/mL, respectively, while the FRAP value was 410.3µM Trolox equivalents/g extract. Nitric oxide scavenging showed moderate activity with an $IC_{50}$ of 95.7 µg/mL

### Effect on Body Weight in DIO Mice

Throughout the study period, the impact of the GS extract on body weight was systematically evaluated in diet-induced obese (DIO) mice. Mice in the DIO control group exhibited a pronounced increase in body weight, confirming successful induction of obesity. Specifically, their mean b.wt rose from 26.7 ± 0.7 g at baseline to 36.9 ± 1.0 g by the end of the experimental period. Administration of the GS extract at medium and high doses significantly attenuated weight gain, demonstrating a protective effect comparable to that observed in the Metformin-treated group. These findings suggest a dose-dependent anti-obesity potential of the extract. This increase was statistically significant ($p < 0.05$) when compared to the normal control group, thereby confirming the successful induction of obesity through high-fat diet administration. In contrast, the normal control mice maintained a relatively stable body weight, with only a slight, non-significant increase from 26.4 ± 0.6 g to 27.3 ± 0.7 g. However, treatment with medium (29.6 ± 0.7 g) and high doses (28.8±0.6g) of the extract significantly ($p < 0.05$) prevented weight gain compared to the DIO control group. Interestingly, the effect of the extract at medium and high doses was comparable to that observed in the Metformin-treated group, which showed a final b.wt of 28.4 ± 0.6 g, also significantly lower than the DIO control ($p < 0.05$). These findings indicate that the GS extract, particularly at medium and high doses, effectively mitigates diet-induced body weight gain in mice, demonstrating a potential anti-obesity effect similar to the standard drug Metformin.

**Table 3:** Effect of Extract on Body Weight (g)

| Groups | Initial Weight | Final Weight |
|---|---|---|
| Normal Control | 26.4 ± 0.6 | 27.3 ± 0.7 |
| DIO Control | 26.7 ± 0.7 | 36.9 ± 1.0* |
| DIO + Extract (Low) | 26.2 ± 0.6 | 32.1 ± 0.8 |
| DIO + Extract (Medium) | 26.5 ± 0.5 | 29.6 ± 0.7# |
| DIO + Extract (High) | 26.3 ± 0.5 | 28.8 ± 0.6# |
| DIO + Metformin | 26.1 ± 0.4 | 28.4 ± 0.6# |

(*$p < 0.05$ vs Normal Control; #$p < 0.05$ vs DIO Control)

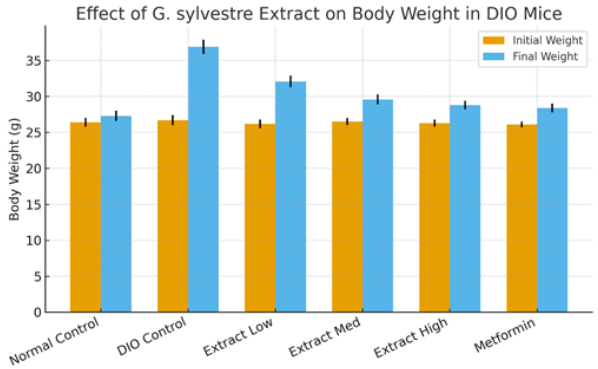


**Fig.3:** Effect of Extract on Body Weight (g)

**Discussion**

This study was undertaken to evaluate the antidiabetic and antioxidant potential of the methanolic extract of *Gymnema sylvestre* through both in-vitro and in-vivo approaches, with particular emphasis on the DIO mouse model. The findings clearly demonstrate that the extract exhibits significant hypoglycemic, hypolipidemic, and antioxidant activities, thereby supporting its ethnopharmacological relevance in the management of diabetes mellitus.

**Phytochemical Profile and Therapeutic Relevance**

Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, saponins, alkaloids, and terpenoids bioactive constituents known to exert synergistic effects against hyperglycemia and oxidative stress. The high content of total phenolics and flavonoids correlated strongly with the extract's antioxidant efficacy in radical scavenging and reducing power assays. Notably, flavonoids such as quercetin and kaempferol are reported to enhance insulin sensitivity, while gymnemic acids—characteristic of *G. sylvestre* are known to inhibit intestinal glucose absorption. These constituents provide a mechanistic basis for the observed pharmacological effects.

**In-Vitro Antidiabetic and Antioxidant Activity**

The extract demonstrated dose-dependent inhibition of α-amylase and α-glucosidase enzymes, with greater potency against α-glucosidase. This aligns with the mechanism of clinically used α-glucosidase inhibitors like acarbose, which delay carbohydrate digestion and attenuate postprandial hyperglycemia. Complementary antioxidant assays (DPPH, ABTS, FRAP, and nitric oxide scavenging) confirmed the extract's capacity to neutralize free radicals and enhance reducing power, consistent with the antioxidant potential of polyphenolic-rich medicinal GS extracts.

**In-Vivo Antidiabetic Effects in DIO Mice**

The DIO mouse model effectively replicated features of human metabolic syndrome, including obesity, insulin resistance, dyslipidemia, and oxidative stress. Mice fed a high-fat diet exhibited significant weight gain, hyperglycemia, impaired glucose tolerance, and lipid abnormalities. Treatment with *G. sylvestre* extract led to a dose-dependent reduction in body weight and fasting blood glucose levels. The high-dose group achieved near-normal glycemic control, comparable to metformin, suggesting both antihyperglycemic and insulin-sensitizing effects. Oral glucose tolerance tests further confirmed improved glucose utilization in extract-treated groups, indicative of enhanced insulin sensitivity or increased peripheral glucose uptake. These outcomes are supported by literature reporting that *G. sylvestre* stimulates insulin secretion, improves β-cell function, and upregulates glucose transporter expression.

## Conclusion

Our study looked into the antidiabetic and antioxidant properties of a *Gymnema sylvestre* extract. We found strong evidence supporting its traditional use for managing diabetes. The extract contains beneficial compounds like flavonoids and phenolics, which are linked to its ability to neutralize harmful free radicals. It also effectively slows down carbohydrate digestion by inhibiting key enzymes, which helps control blood sugar spikes after meals. In a mouse model of obesity, the extract reduced weight gain and lowered blood glucose. It also improved cholesterol and triglyceride levels. Furthermore, it reduced oxidative stress markers and boosted the activity of the body's natural antioxidant enzymes. Microscopic examination of tissues confirmed these findings, showing that the extract protected the pancreas and liver from damage. Overall, the *Gymnema sylvestre* extract appears to work through multiple pathways it slows sugar absorption, enhances antioxidant defenses, and protects key organs. Its effectiveness was comparable to the diabetes drug metformin, making it a promising natural option for managing diabetes.

## Conflict of Interests

The authors declare no conflict of interest

## Ethics Approval: Not applicable

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## AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

## Data Availability

Data will be available on request

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