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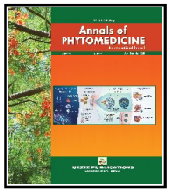

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In vitro and clinical investigation of the antidiabetic properties of *Gymnema sylvestre* R. Br. leaf extract

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Abstract

Chronic hyperglycemia is a characteristic of type 2 diabetes mellitus (T2DM), a well-known metabolic disease. For complementary diabetes management, it has been common practice to use plant-based remedies such as *Gymnema sylvestre* R. Br., which has been traditionally utilized for reducing blood sugar. The present research aims to investigate the antidiabetic properties of *G. sylvestre* leaf extract through *in vitro* cellular assessment and a clinical trial study. *In vitro*, *G. sylvestre* leaf extract was tested for glucose uptake in 3T3-L1 adipocytes and insulin secretion in MIN-6 ß-cells at varying concentrations. Clinically, an open-label trial was conducted on adults with type 2 diabetes (n = XX), assigned to experimental (2 g/day leaf powder) and control groups for 45 days. Pre- and post-intervention anthropometric and biochemical parameters were measured. Data were analyzed statistically (p<0.05). *G. sylvestre* extract enhanced glucose uptake in 3T3-L1 adipocytes (r = –0.98, p<0.01) and increased insulin secretion in MIN-6 cells by ~50% at 750 µg/ml (p<0.001). Clinically, 45-day supplementation (2 g/day) reduced body weight (–4.17%), BMI (–4.19%), waist-hip ratio (–3.06%), fasting glucose (–27.17%), random glucose (–20.47%), and HbA1c (–6.38%) (p<0.01), with no significant changes in controls. *G. sylvestre* leaf extract demonstrates significant antidiabetic potential by enhancing glucose uptake and insulin secretion *in vitro* and improving anthropometric and glycemic indices in clinical subjects, supporting its role as a complementary therapeutic agent for diabetes management.

1. Introduction

Chronic hyperglycemia is a typical characteristic of diabetes mellitus, a metabolic condition that can cause serious complications affecting essential organs (Kaur *et al.*, 2011). Worldwide, 537 million people between the age of 20-79 years are estimated to be suffering from diabetes, and by 2030, that figure is expected to increase to 643 million (IDF Diabetes Atlas, 2021).

The way that dietary carbohydrates are absorbed and digested in the small intestine depends on the food's physical form and botanical origin (Kaur *et al.,* 2011). Consequently, the type of food consumed affects postprandial insulin and blood glucose levels. Diets high in rapidly digestible carbohydrates cause sharp spikes in blood glucose and insulin, which can have detrimental effects on health (Singh *et al.,* 2010). A more gradual rise in blood glucose is considered beneficial for both healthy individuals and those with diabetes. Thus, dietary recommendations for diabetic patients typically focus on consuming foods rich in slowly digestible carbohydrates and avoiding sugar and sugary foods. However, specific guidance on incorporating foods with hypoglycemic properties is generally lacking (Kaur *et al.,* 2011).

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Despite the number of antidiabetic drugs have been developed, such as insulin therapy, acarbose, metformin, dopamine agonists, DPP-4 inhibitors, glucagon-like peptides, SGLT-2 inhibitors, and miglitol (alpha-glucosidase inhibitors), many of these treatments have serious side effects that may prevent them from being used for an extended period of time (Haq *et al.,* 2021). This has led to an increase in interest in safer and more affordable antidiabetic alternatives.

India, which has the highest number of diabetic patients globally, has a long tradition of using certain vegetables and medicinal plants recognized for their antidiabetic properties, potentially offering natural adjuncts for diabetes management (Kaur *et al.,* 2011). Various plant-derived compounds, including alkaloids, glycosides, carbohydrates, and steroids, have demonstrated therapeutic potential towards management of T2DM (Bailey and Day, 1989). In recent years, the use of traditional plant-based remedies has increased due to their natural origin, affordability, and reduced risk of adverse effects (Pal *et al.,* 2021). Medicinal plants like bitter melon, garlic, ginger, roselle *etc*., are commonly used for their hypoglycemic properties (Yedjou *et al.,* 2023).

Among these, *G. sylvestre* has been widely reported to exhibit significant antidiabetic activity (Kanetkar *et al.,* 2007; Khan *et al.,* 2019; Kashif *et al.,* 2023). The bioactive compounds in *G. sylvestre*, such as gymnemic acids, have been shown to stimulate insulin secretion, regenerate pancreatic β-cells, and reduce glucose absorption in the intestine, thereby improving glycemic control (Persaud *et al.,* 1999; Baskaran *et al.,* 1990). However, much of the existing research relies on animal models or crude extracts, with limited clinical data substantiating its efficacy in humans.

This study attempts to assess the antidiabetic effects of *G. sylvestre* leaf extract using a combination of *in vitro* and clinical methodologies, given the rising incidence of diabetes and the encouraging initial results. Specifically, *in vitro* assays were conducted using 3T3-L1 adipocytes and MIN-6 pancreatic β-cell lines to investigate glucose uptake and insulin secretion, respectively. In order to evaluate the clinical efficacy of *G. sylvestre* supplementation on anthropometric parameters and diabetic biomarkers, a concurrent *in vivo* clinical trial involving male type 2 diabetic patients was conducted. Gymnemic acid which is the main bioactive ingredient in *G. sylvestre,* has shown to have potent antidiabetic effect. However, in order to preserve the synergistic effects of other phytochemicals like flavonoids, tannins, and saponins that may improve antidiabetic efficacy, the current study used a crude aqueous extract. Additionally, using crude dried leaf powder for the *in vivo* clinical application guaranteed dose practicality, stability, and conformity to conventional formulations, promoting both translational relevance and scientific rigor.

This integrated approach addresses limitations of prior studies by providing mechanistic insights at the cellular level alongside clinically relevant outcomes. The findings are expected to contribute novel evidence supporting the use of *G. sylvestre* as a safe, effective, and affordable therapeutic adjunct in diabetes management, with potential implications for dietary recommendations and phytopharmaceutical development.

2. Materials and Methods

2.1 Plant authentication

This plant was authenticated by Professor, Department of Botany, Osmania University, Hyderabad, T.S. Herbarium Number is :

2.2 Research involving human participants and/or animals

Ethical approval: The study was approved by the University Ethical Committee of Punjab Agricultural University (Memo No. DR.III.AU.2021/10782-98). Informed consent was obtained from all participants. No animal studies were conducted; hence, CPCSEA guidelines were not applicable.

2.3 Materials

2.3.1 *G. sylvestre* leaves

Leaves of *G. sylvestre*, commonly known as Madhunashini or Gurmar, were locally collected, multiplied, and cultivated by the Herbal Garden of Punjab Agricultural University (PAU), Ludhiana. The plant was identified based on its morphological characteristics and standard taxonomic features, and is recognized and maintained by the University's Botanical experts. No formal Botanical Authentication Number was issued; however, the plant material is institutionally recognized and maintained for research and educational purpose.

After thoroughly cleaning the leaves with tap water to get rid of dirt and surface contaminants, they were rinsed with distilled water. After cleaning, the leaves were dried at 50°C in a hot-air oven until they were totally dehydrated. After that, a mortar and pestle were used to grind the dried leaves into a fine powder. The powdered G. sylvestre leaves were kept at 5°C in airtight containers until further use.

2.3.2 Cell lines

The 3T3-L1 mouse preadipocyte cell line (ATCC® CL-173™, RRID: CVCL_0123) and the MIN-6 murine pancreatic β-cell line (RRID: CVCL_0431) were used in this study. Both cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. The selection of 3T3-L1 and MIN-6 lines was based on their extensive validation in glucose uptake and insulin secretion studies, respectively (Zhou *et al*., 2007; Liu *et al*., 2009)

A contamination free cell suspension was cultured in 25 cm^2 cell culture flasks and incubated for 48-72 h at 37°C. After 2-3 days, cells were sub-cultured to another 25 cm^2 flask by trypsinization. Before trypsinization, cells were washed with PBS twice and added 1000 µl TPGV solution. Gently pipetting was done to form a uniform suspension. Fresh sterile 25 cm^2 flasks were seeded with the cells at a split ratio of 1:2 and 5 ml of GM (growth media) was added and incubated at 37°C in a growth chamber with controlled humidity and CO^2 level.

2.4 Biochemical analysis

2.4.1 Mineral composition analysis

The mineral profile of *G. sylvestre* leaf powder was assessed using Atomic absorption spectrophotometry (AAS) as per standard protocol by Piper (1950). Dried leaf samples were first subjected to acid digestion using a concentrated mixture of nitric acid and perchloric acid (3:1 ratio) in a digestion chamber until a clear solution was obtained. The digested samples were then filtered and diluted with deionized water to a fixed volume.

The concentrations of essential minerals including iron (Fe), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), and potassium (K) were determined using a calibrated AAS system (AAS, Varian model). Phosphorus (P) content was estimated colorimetrically using the vanado-molybdate method, and sodium (Na) levels were quantified using a flame photometer. All measurements were carried out in triplicate. The results were expressed in mg per 100 g (mg/100 g) of dry weight of the leaf powder

2.4.2 Bioactive compounds and antioxidant activity

2.4.2.1 Extraction of bioactive compounds

Dried *G. sylvestre* leaf powder (1 g) was extracted with 15 ml of 80% methanol acidified to pH 2.0 using 6N HCl. The mixture was shaken for 30 min at room temperature and re-extracted under identical conditions. The pooled supernatants were centrifuged (6000 rpm, 15 min), filtered through Whatman No. 1 paper, and the final volume was adjusted to 50 ml with 80% methanol. Extracts were stored at –20°C until further use in phenolic and antioxidant assays.

2.4.2.2 Total phenolic content (TPC)

TPC was determined using the Folin - Ciocalteu method (Singleton *et al*., 1999). Briefly, 0.5 ml of extract was mixed with 2.5 ml of 10% (v/v) Folin - Ciocalteu reagent, followed by 2.0 ml of 7.5% (w/v) sodium carbonate. After incubation in the dark for 30 min at room temperature, absorbance was read at 765 nm on a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight.

2.4.2.3 Total flavonoid content (TFC)

TFC was measured using the aluminium chloride colorimetric assay (Zhishen *et al*., 1999). The reaction mixture contained 0.5 ml of extract, 1.5 ml methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml distilled water. After incubation for 30 min at room temperature, absorbance was recorded at 510 nm. Results were expressed as mg rutin equivalents (RE) per 100 g dry weight.

2.4.2.4 DPPH radical scavenging activity

The antioxidant activity was assessed using the DPPH assay (Brand-Williams *et al*., 1995; Tadhani *et al*., 2007). One milliliter of extract was added to 2 ml of 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark for 30 min at room temperature, and absorbance was measured at 517 nm. Activity was calculated using a Trolox calibration curve and expressed as mg Trolox equivalents (TE) per 100 g dry weight.

2.4.2.5 Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed as per Benzie and Strain (1999) and modified by Tadhani *et al*. (2007). The FRAP reagent (300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl*f* •6H, O in a 10:1:1 ratio) was freshly prepared. To 3 ml of reagent, 100 µl of extract was added and incubated for 30 min at 37°C. Absorbance was measured at 593 nm. Results were expressed as mg TE/100 g dry weight.

2.4.2.6 Reducing power assay

Reducing power was determined by the potassium ferricyanide method (Oyaizu, 1986). Reaction mixtures contained 2.5 ml of 0.2 M phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of extract. Samples were incubated at 50°C for 20 min, followed by the addition of 2.5 ml of 10% trichloroacetic acid. After centrifugation (3000 rpm, 10 min), the upper layer was diluted with distilled water, and absorbance was read at 700 nm.

2.4.2.7 ABTS radical scavenging activity

The $ABTS^{\bullet+}$ assay was performed following Re *et al*. (1999). The ABTS radical cation was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12-16 h. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. One milliliter of extract was added to 2 ml of ABTS solution, and absorbance was recorded after 6 min at 734 nm. Results were expressed as mg TE/100 g dry weight.

2.5 Leaf extract preparation

The preparation of *G. sylvestre* leaf extract using the infusion method was standardized as per the protocol described by Gray and Flatt (1997). Twenty grams of leaf powder was suspended in 50 ml of distilled water, boiled for 15-20 minutes, and reduced to a thick suspension of approximately 20 ml. The suspension was then filtered through Whatman No. 1 filter paper, and the volume was adjusted again to 20 ml using distilled water. The extract was freeze-dried, and the yield was calculated on a dry-weight basis. The freeze-dried yield was 9% (w/w) of the starting material (1.8 g per ml of leaf extract). The powder was stored at –20°C for future use. For larger-scale production, the quantities of ingredients were proportionally increased while adhering to the standardized protocol.

2.6 *In vitro* glucose uptake assay

The effect of the aqueous preparation of *G. sylvestre* leaf extract powder on glucose uptake by 3T3-L1 cells was evaluated following Zhou *et al*. (2007).

2.6.1 Differentiation of 3T3-L1 cells

To assess glucose uptake, 3T3-L1 cells were first differentiated into adipocytes using, the Zhou *et al*. (2007) protocol, with minor adjustments. DMEM medium supplemented with 10% newborn calf serum (NCS) and 25 mM glucose was used to cultivate the cells. To induce adipocyte differentiation, the medium was changed to DMEM containing 10% NCS, 250 nM dexamethasone, 0.5 mM 1-methyl-3-isobutylxanthine, and 1 µg/ml insulin at 80% confluency. The mixture was then incubated for 48 h.

Differentiated 3T3-L1 cells were cultured in 96-well plates with DMEM and 0.2% BSA, preincubated for 12 h, and then treated with varying concentrations (100-2000 µg/ml) of the aqueous preparation of leaf extract powder for 24 h. Glucose concentrations were measured using the GOD-POD method (Shaker and Swift, 2024). Microscopic observations were conducted to assess cytological changes in both control and treated cells.

2.7 *In vitro* insulin secretion assay

The effect of the aqueous preparation of *G. sylvestre* leaf extract on insulin secretion by MIN-6 pancreatic β-cells was assessed following Kaur *et al*. (2011), with modifications. MIN-6 cells were cultured in DMEM supplemented with 10% NCS, seeded at 30,000 cells per well in a 96-well plate, and pre-incubated for 2 h in a physiological salt solution containing 2 mM glucose. Cells were then exposed to varying concentrations (100-2000 µg/ml) of the aqueous preparation of leaf extract powder. Insulin secretion was measured using a radioimmunoassay (Liu *et al.* 2009). Microscopic observation was conducted to assess cytological changes in both control and treated cells.

2.8 *In vivo* experiment on diabetic patients

A 45-day *in vivo* study was conducted on 60 adults (30-50 years) with type 2 diabetes. Inclusion criteria were fasting blood glucose 125-150 mg/dl, HbA_1c 6-7%, and absence of medical complications. Exclusion criteria included insulin therapy, comorbid disorders (cardiovascular, renal, or hepatic), recent use of herbal supplements, smoking, or alcohol abuse. Restricting participants to a single gender with mild to moderate diabetic status minimized physiological variability and avoided confounding from advanced complications.

For the clinical assessment, thirty patients each were split into the experimental (E) and control (C) groups. In terms of weight, and waist-to-hip ratio (W/H ratio), the two groups were nearly identical. The experimental group received a dose of 2 g per day of *G. sylvestre* leaf powder, which was derived from human equivalent dosages extrapolated from previous studies (Paliwal *et al.,* 2009; Nanda *et al.,* 2010) that showed glycemic improvements at similar doses (500 mg-6 g/day. The dose of 2 g/day of *G. sylvestre* leaf powder administered to the experimental group was based on human equivalent dosages extrapolated from earlier studies (Paliwal *et al.,* 2009; Nanda *et al.,* 2010), which demonstrated glycemic improvements at comparable doses (500 mg-6 g/day). The baseline and endline of the experimental period were used to measure

anthropometric parameters, such as changes in body weight, body mass index (BMI), and W/H ratio. An Accu-Chek glucometer, the Folin-Wu method (Oser, 1976), and the ion exchange resin method (Davis *et al.,* 1978) were also used to measure diabetic-related biomarkers, such as fasting blood glucose (FBG), random blood glucose, and HbA_1c levels.

2.8.1 Rationale for experimental models and dosage

The 3T3-L1 mouse preadipocyte and MIN-6 murine pancreatic β-cell lines were employed as validated *in vitro* models for adipocyte glucose uptake and pancreatic β-cell insulin secretion, respectively. 3T3-L1 cells closely mimic adipocyte physiology and are widely used for glucose transport studies, while MIN-6 cells are a well-established model for insulin release (Zhou *et al*., 2007; Liu *et al*., 2009). For the clinical study, the intervention dose of 2 g/day *G. sylvestre* leaf powder was selected based on human equivalent dosages extrapolated from earlier trials (Paliwal *et al*., 2009; Nanda *et al*., 2010), which demonstrated significant glycemic improvements at 500 mg-6 g/day without adverse effects. A crude extract was used rather than isolated gymnemic acid, as the synergistic action of multiple bioactive constituents (saponins, flavonoids, tannins, and phenolics) may better represent the plant's traditional therapeutic use and enhance translational relevance.

2.9 Statistical analysis

All statistical analyses were performed using SPSS version 16. Descriptive statistics (mean ± standard deviation and percentage change) were calculated for all variables. Paired t-tests were applied to compare baseline and post-intervention values within each group, while independent t-tests were used for between-group comparisons. Pearson's correlation analysis was performed to evaluate the relationship between extract concentration and glucose uptake *in vitro*. A linear regression trendline was plotted to illustrate the dose-response relationship. Statistical significance was set at $p<0.05$.

3. Results

3.1 Biochemical properties of *G. sylvestre* leaf extract

The biochemical profile of *G. sylvestre* leaf extract highlights its therapeutic potential in diabetes management. Gymnemic acid is the principal bioactive constituent of *G. sylvestre*. Structurally analogous to glucose, gymnemic acid competitively inhibits intestinal glucose absorption by interacting with glucose transporters, thereby reducing postprandial hyperglycemia (Yoshikawa *et al*., 1993; Parveen *et al*., 2016). Furthermore, gymnemic acid has been reported to stimulate insulin secretion and promote pancreatic β-cell regeneration, contributing to improved glycemic control (Kanetkar *et al*., 2007; Persaud *et al*., 1999). While this mechanistic model provides a summarized pathway for its antidiabetic action, further studies using advanced molecular imaging and receptor-ligand interaction analyses are required for validation.

Quantitative mineral analysis of the powdered leaf extract indicated notable levels of zinc (4.62 mg/100 g), calcium (1095.11 mg/100 g), magnesium (652.86 mg/100 g), and iron (35.13 mg/100 g). Phytochemical profiling revealed a rich content of total flavonoids (2145.22 mg rutin equivalent [RE]/100 g) and total phenolics (288.97 mg gallic acid equivalent [GAE]/100 g). Gymnemic acid, the principal bioactive constituent, was obtained with a yield of 65.1%, confirming its dominant presence in the extract.

The extract exhibited significant antioxidant potential, as demonstrated by DPPH radical scavenging activity (459.34 mg Trolox equivalent [TE]/100 g), ABTS radical scavenging activity (11.44 mg TE/100 g), and ferric reducing antioxidant power (FRAP; 683.59 mg TE/100 g). Collectively, these findings underscore the extract's capacity to neutralize free radicals, which may contribute to the mitigation of oxidative stress associated with diabetes.

3.2 *In vitro* glucose uptake analysis

Differentiated 3T3-L1 adipocytes were exposed to increasing concentrations of *G. sylvestre* leaf extract (100-2000 µg/ml) to evaluate the extract's capacity to absorb glucose. Table 1 shows a concentration-dependent decrease in extracellular glucose concentration. The lowest glucose level (53.63 ± 1.58 mg/dl) was recorded at 2000 µg/ml, which was significantly lower than the untreated control (95.63 ± 1.18 mg/dl, $p<0.001$).

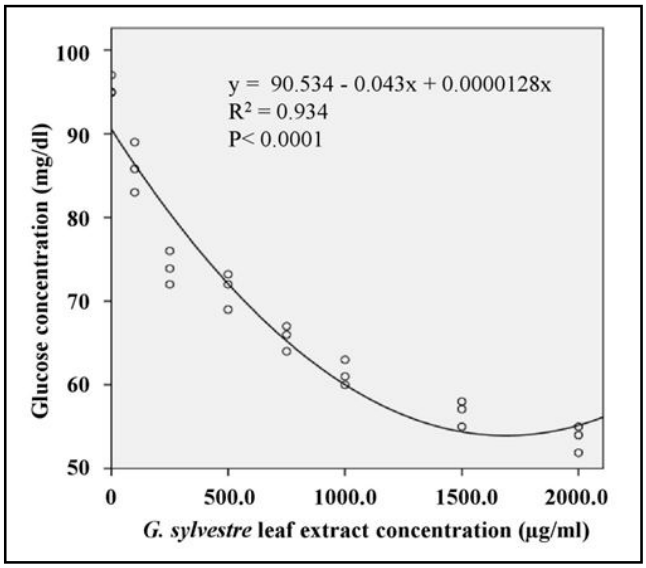

Figure 1: Effect of *G. sylvestre* leaf extract on glucose uptake by 3T3-L1 adipocytes, demonstrating dose-dependent enhancement of glucose absorption.

Table 1: Effect of *G. sylvestre* leaf extract powder on glucose uptake by 3T3-L1 cells

G. sylvestre leaf extract powder (µg/ml of media)	Glucose concentration (mg/dl) (Mean ± SD)
Control	95.63 ± 1.18
100,0	85.93 ± 9.0
250.0	73.96 ± 2.0
500.0	71.40 ± 2.16
750.0	65.66 ± 1.52
1000.0	61.33 ± 1.52
1500.0	56.70 ± 1.53
2000.0	53.63 ± 1.58

SD: Standard deviation

Figure 1 illustrates the significant inverse relationship between the extract concentration and the media's glucose concentration (Pearson's $r = –0.98$, $p<0.01$). Particularly at higher doses, microscopic images

(Figure 2) indicated increased cellular density in treated wells. Even though morphological changes indicated a possible increase in cell density after treatment, conclusive results are not possible due to the absence of proliferation-specific assays.

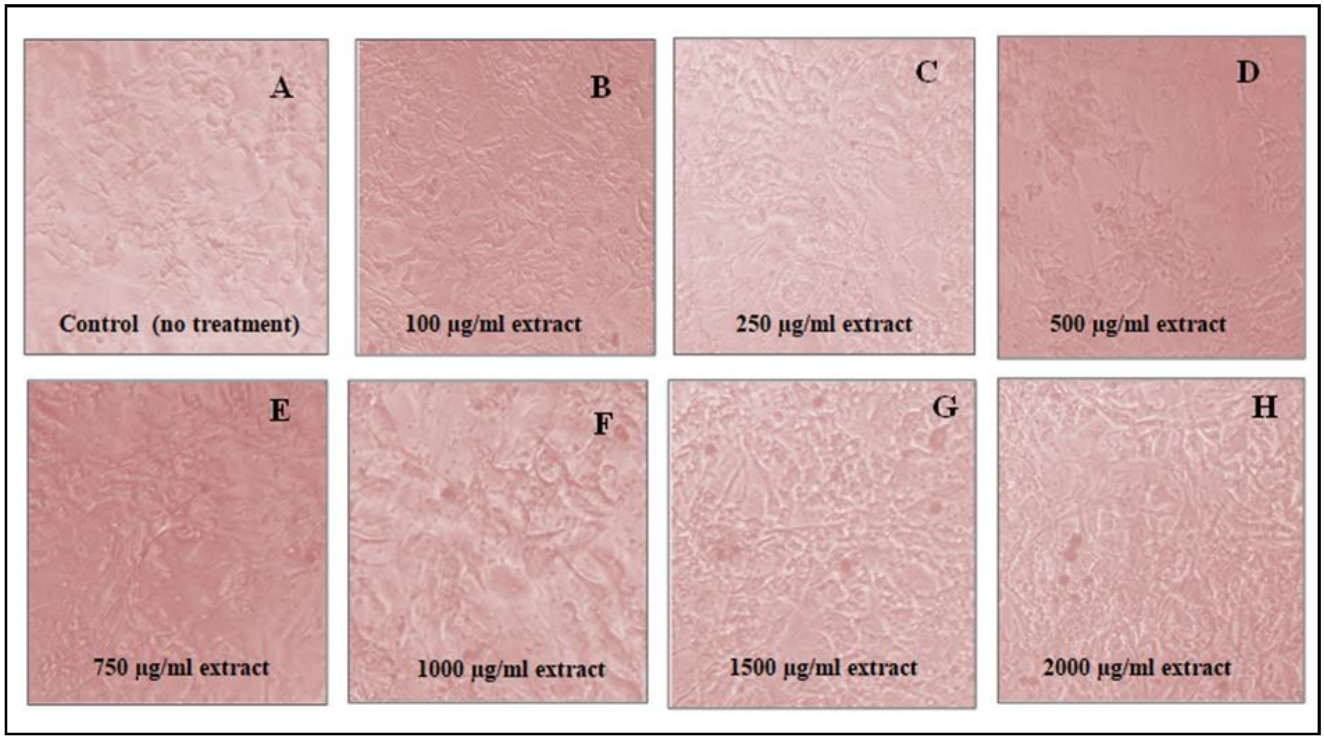

Figure 2: Microscopic examination of 3T3-L1 cells: control (A), and cells treated with *G. sylvestre* leaf extract at concentrations of 100 µg/ml (B), 250 µg/ml control, 500 µg/ml (D), 750 µg/ml (E), 1000 µg/ml (F), 1500 µg/ml (G), and 2000 µg/ml (H).

3.3 *In vitro* insulin secretion analysis

MIN-6 pancreatic β-cells cultured at basal glucose levels (2 mM) were used to test the impact of *G. sylvestre* leaf extract on insulin secretion. Up until 750 µg/ml, insulin levels rose in a dose-dependent manner. At this point, the peak reduced cell viability was indicated by morphological changes, including cell shrinkage, detachment, and membrane irregularities, that were seen at ≥1000 µg/ml (Figure 3)

Table 2: Effect of aqueous preparation of *G. sylvestre* leaf extract powder on insulin secretion by MIN-6 cells

G. sylvestre leaf extract powder (µg/ml of media)	Insulin Secretion (µU/l) (Mean ± SD)
Control	24.53 ± 1.15
100.0	26.94 ± 0.05
250.0	31.00 ± 1.50
500.0	35.00 ± 0.20
750.0	36.75 ± 0.75
1000.0	33.10 ± 0.90
1500.0	31.22 ± 0.76
2000.0	30.47 ± 1.39

SD: Standard deviation

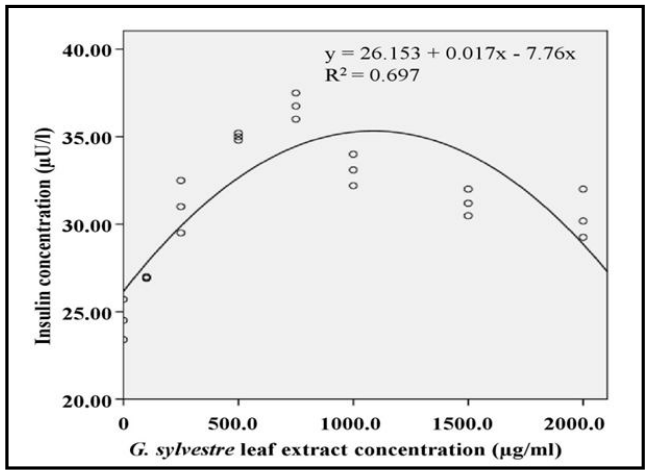

Figure 3: Effect of *G. sylvestre* leaf extract on insulin secretion by MIN-6 pancreatic β-cells, indicating a concentration-dependent increase in insulin release.

Insulin secretion was 36.75 ± 0.75 µU/l, which was much higher than the control (24.53 ± 1.15 µU/l, $p</ 0.001$). However, insulin secretion decreased at concentrations greater than 750 µg/ml, indicating potential cytotoxic effect at higher doses (Table 2, Figure 3). However, no cytotoxicity tests (such as MTT or LDH release) were carried out to validate these findings. Furthermore, given that the extract is frequently used in humans at doses of up to 2 g/day, therefore toxicological evaluations were not included in the study.

Higher extract concentrations may cause morphological changes in MIN-6 cells, which could indicate underlying mechanisms like calcium dysregulation or ER stress. However, the current study had no molecular or imaging assays to support these pathways.

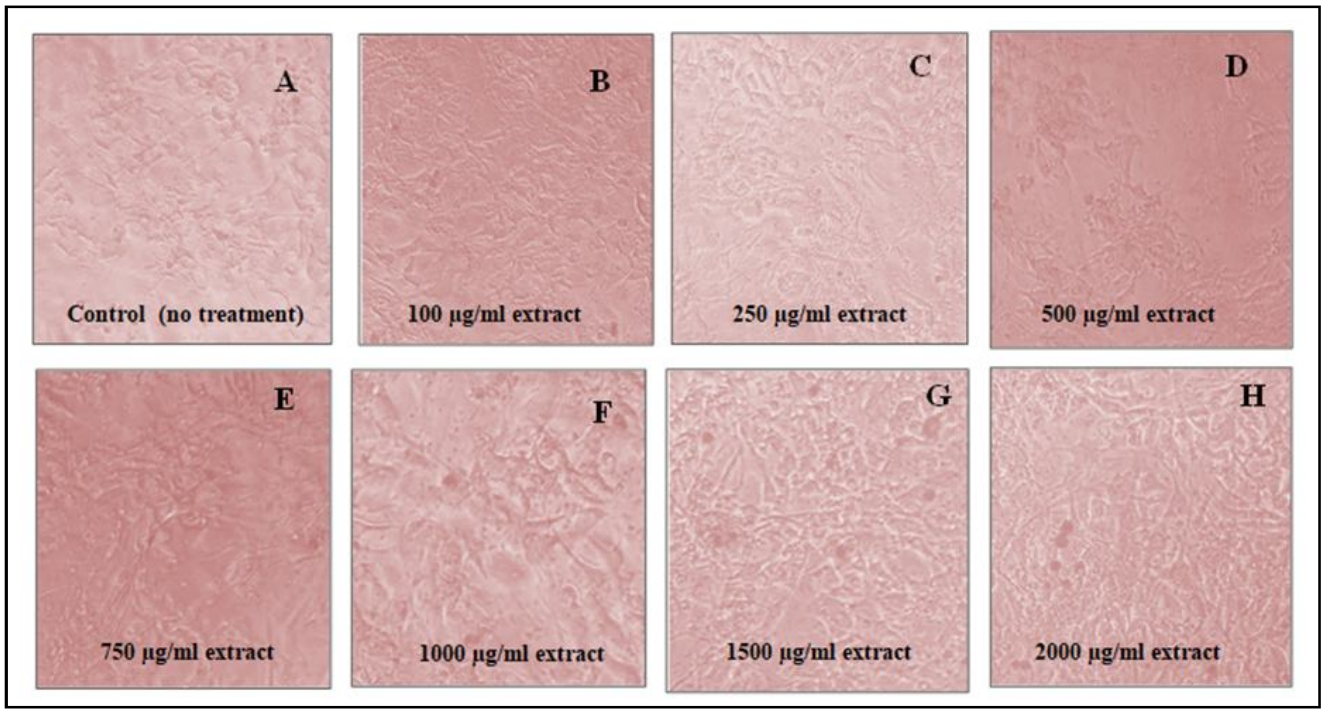

Figure 4: Microscopic examination of MIN-6 cells : control (A), and cells treated with *G. sylvestre* leaf extract at concentrations of 100 µg/ml (B), 250 µg/ml control, 500 µg/ml (D), 750 µg/ml (E), 1000 µg/ml (F), 1500 µg/ml (G), and 2000 µg/ml (H).

3.4 *In vivo* evaluation of antidiabetic potential of *G. sylvestre* leaf extract

The 45-day *in vivo* evaluation involved two groups: Group E (Experimental), which was given an oral dosage of 2 g/day of powdered *G. sylvestre* leaf extract, and Group C (Control), which was not given the extract. The treatment potential of *G. sylvestre* extract was evaluated by tracking glycemic biomarkers and anthropometric indices.

Table 3: Anthropometric characteristics of control and experimental groups pre- and post-intervention

Indices	Group	Baseline	Endline	% Change	t-value
Height (cm)	C	166.28 ± 8.63	166.28 ± 8.63	-	-
	E	167.54 ± 0.08	167.54 ± 0.08	-	-
	t-test	0.56^{NS}	0.56^{NS}		
Weight (kg)	C	077.08 ± 10.73	076.94 ± 9.50	-0.18	2.33^{**}
	E	073.25 ± 8.10	070.19 ± 8.20	-4.17	11.27^{**}
	t-test	1.56^{NS}	2.15^{*}		
BMI (kg/m^2)	C	027.97 ± 4.01	027.90 ± 3.75	-0.25	0.59^{NS}
	E	026.14 ± 2.69	025.04 ± 2.71	-4.19	10.87^{**}
	t-test	2.07^{*}	2.65^{**}		
W/H Ratio	C	000.97 ±0.08	000.97 ± 0.11	-	0.07^{NS}
	E	000.98 ± 0.07	000.95 ± 0.04	-3.06	10.29^{**}
	t-test	0.09^{NS}	7.52^{*}		

SD: Standard deviation
**Significant at 1% level of significance ($p<0.01$)
*Significant at a 5% level of significance ($p<0.05$)
^{NS}: Nonsignificant

3.4.1 Anthropometric parameters

The mean height, weight, BMI, and waist-to-hip (W/H) ratio did not differ statistically significantly among the two groups at baseline (Table 3). However, at the end of the intervention, the Experimental group was shown to have significant reduction in the body weight going from 73.25 ± 8.10 kg to 70.19 ± 8.20 kg, a decrease of 4.17% (p<0.01). On the other hand, control group observed a non-significant 0.18% (p>0.05) decrease in weight, from 77.08 ± 10.73 kg to 76.94 ± 9.50 kg.

BMI values in Group E decreased significantly from 26.14 ± 2.69 kg/m² to 25.04 ± 2.71 kg/m², showing 4.19% of reduction (p<0.01). Conversely, Group C exhibited a non-significant change (27.97 ± 4.01 to 27.90 ± 3.75 kg/m²; p > 0.05). The W/H ratio in Group E decreased significantly by 3.06% (0.98 ± 0.07 to 0.95 ± 0.04; p<0.01), while no significant changes were observed in Group C (0.97 ± 0.08 to 0.97 ± 0.11; p>0.05). These findings indicate that supplementation of *G. sylvestre* leaf extract may contribute to beneficial anthropometric alterations, particularly reductions in central adiposity and BMI.

3.4.2. Glycemic biomarker profile

Group C showed a non-significant decrease of 1.32% (138.8 ± 8.71 to 136.97 ± 12.17 mg/dl; p>0.05), whereas experimental group supplemented with leaf powder observed significantly decrease in fasting blood glucose (FBG) levels from 139.6 ± 10.34 mg/dl to 101.67 ± 11.17 mg/dl, or a 27.17% reduction (p<0.01) (Table 4). At the endpoint, the between-group comparison revealed a highly significant difference (p<0.01). Random blood glucose (RBG) showed a similar trend, declining significantly by 20.47% in Group E (233.97 ± 18.9 to 186.07 ± 21.2 mg/dl; p<0.01) while Group C showed no significant change (232.57 ± 18.8 to 231.86 ± 20.4 mg/dl; p > 0.05).

Glycated hemoglobin (HbA_1c) in Group E decreased from 6.73 ± 0.44% to 6.30 ± 0.45%, representing a 6.38% reduction (p<0.01). Although statistically significant, this decrement may not be sufficient to restore normoglycemia, as post-intervention HbA_1c levels remained above the ICMR-recommended threshold for euglycemia (<5.7%). In contrast, Group C demonstrated a non-significant increase in HbA_1c (6.63 ± 0.44% to 6.65 ± 0.45%).

Table 4: Glycemic biomarker profile of control and experimental groups pre- and post-intervention

Biomarkers	Groups	Baseline#	Endline#	% Change	t-value
Fasting blood glucose	C	138.80 ± 8.71	136.97 ± 12.17	-1.32	0.873^{NS}
(mg/dl)	E	139.60 ± 10.34	101.67 ± 11.17	-27.17	17.71**
(70-100 mg/dl)[a]	t-test	0.32^{NS}	11.7**		
Random blood glucose	C	232.57 ± 18.8	231.86 ± 20.4	-0.3	0.196 ^{NS}
(mg/dl)	E	233.97 ± 18.9	186.07 ± 21.2	-20.47	12.32**
(< 200 mg/dl)[a]	t-test	0.28^{NS}	8.54**		
Glycated haemoglobin	C	006.63 ± 0.44	006.65 ± 0.45	0.27	1.19 ^{NS}
(%)(< 5.7%)[a]	E	006.73 ± 0.44	006.30 ± 0.45	-6.38	7.98**
	t-test	0.85^{NS}	3.01**		

SD: Standard deviation
[a](ICMR, 2018)
^{NS}Nonsignificant
**Significant at 1% level (p<0.001)

4. Discussion

The most common feature of type 2 diabetes mellitus (T2DM), a worldwide health concern, is persistent hyperglycemia brought on by insulin resistance and/or decreased insulin secretion. Conventional antidiabetic medications, while effective, are often accompanied by adverse effects, high costs, and limited long-term compliance. Alternative therapies, particularly herbal remedies that have been used historically in traditional medical systems like Ayurveda and Traditional Chinese Medicine (TCM), are therefore gaining popularity (Wang *et al.,* 2013). *G. sylvestre*, also known as "gurmar" or the "sugar destroyer," is one of these that has demonstrated encouraging therapeutic potential because of its distinct pharmacological profile.

Gymnemic acids, which competitively block intestinal glucose absorption and alter sweet taste receptors, are primarily responsible for *G. sylvestre*'s bioactivity (Tiwari *et al.,* 2014). The plant's hypolipidemic, antioxidant, and anti-inflammatory qualities, in addition to its glycemic effects, may help explain its wider metabolic advantages, which include lowered appetite, better lipid profiles, and control of body weight (Turner *et al.,* 2022). To determine the antidiabetic potential of *G. sylvestre* leaf extract powder, a combined in vitro and clinical evaluation was conducted in the current study. The purpose of using crude extract, as opposed to studies that only looked at isolated compounds, was to preserve a wide range of bioactive phytoconstituents, including flavonoids, saponins, and tannins, which may work in concert to improve the overall therapeutic effect (Gupta *et al.,* 2012; Pal *et al.,* 2021).

In vitro glucose uptake was measured using differentiated 3T3-L1 adipocytes. These cells are frequently employed as models for studying glucose metabolism because they closely resemble adipogenic differentiation and insulin sensitivity (Zhou *et al*., 2007). *G. sylvestre* extract treatment resulted to a statistically significant, dose-dependent increase in glucose uptake (p<0.01). According to earlier research, different antidiabetic botanicals have been shown to lower blood sugar levels in comparable models (Kaur *et al.,* 2011). Improved cellular glucose utilization and metabolic support from the extract may be the cause of the increased proliferation observed under microscope.

MIN-6 cells were used to measure insulin secretion because they are derived from mouse pancreatic β-cells and maintain glucose-responsive insulin secretion that is comparable to that of native islets (Ishihara *et al.,* 1993). Insulin secretion increased significantly in response to the extract up to 750 µg/ml, after which it began to decline. This biphasic reaction points to a threshold that, at higher concentrations, may cause morphological changes and decreased cell viability, which could indicate cytotoxicity or endoplasmic reticulum (ER) stress (Liu *et al.,* 2009; Fu *et al.,* 2012). At supra-physiological concentrations, the decrease in insulin secretion may be explained by the impairment of β-cell function caused by elevated cytosolic calcium and ER stress.

The clinical trial of this study included 60 male T2DM participants receiving 2 g/day of *G. sylvestre* leaf powder for 45 days. The treatment group showed significant improvements in anthropometric and metabolic parameters. The observed average weight loss of 4.17 kg, compared to 0.18 kg in controls, was substantial and exceeds seasonal variations in weight reported by Yoshimura *et al*. (2020). This supports the satiety-enhancing and metabolic effects previously described in human studies (Woodgate and Conquer, 2003). Additionally, reductions in BMI and waist-to-hip ratio align with the findings of Basciani *et al.* (2023), further supporting the metabolic benefits of *G. sylvestre* supplementation.

Significant decreases in HbA_1c (6.4%), random blood glucose (20.4%), and fasting blood glucose (27.2%) were shown among the experimental group, suggesting better glycemic control. These findings support previous preclinical research showing antihyperglycemic effects in rodent models (Kashif *et al.,* 2023; Aralelimath and Bhise, 2012). The significant decrease from 6.73% to 6.30% ($p<0.01$) over a relatively short period (45 days) is clinically meaningful and indicates that a longer-term intervention could yield more normalized values, even though post-intervention HbA_1c levels remained slightly above the optimal range (<5.7%; ICMR, 2018). Given that HbA_1c represents average blood glucose levels over an 8-12 week period, this slight change may have been caused by the intervention's relatively short duration, which may not have been sufficient for completely capturing *G. sylvestre's* glycemic-lowering potential.

5. Conclusion

The current study offers comprehensive evidence of antidiabetic potential of *G. sylvestre* leaf extract powder by means of a clinical trial as well as in vitro mechanistic investigations. The extract showed dual-action antidiabetic properties by stimulating insulin secretion from pancreatic β-cells and improving glucose uptake in adipocytes. In clinical trial, it contributed to weight loss, improved anthropometric indices, and a significant improvement in glycemic parameters. Additionally, the utilization of crude extract instead of isolated gymnemic acid made it possible for several phytochemicals to interact synergistically, increasing the effectiveness of treatment. These results demonstrate the value of *G. sylvestre* in integrative diabetes management strategies, especially for individuals seeking complementary, alternative therapies.

Further long-term, placebo-controlled clinical trials with larger sample sizes and detailed pharmacokinetic analyses are warranted to validate these findings, identify optimal dosing strategies, and assess safety profiles. Nonetheless, this study adds to the growing body of evidence supporting the use of traditional herbal medicines like *G. sylvestre* in combating the rising global burden of type 2 diabetes mellitus.

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List of abbreviations (Non-standard)

- AAS: Atomic Absorption Spectrophotometry
- ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
- DPPH: 2,2-Diphenyl-1-picrylhydrazyl
- DMEM: Dulbecco's Modified Eagle Medium
- FRAP: Ferric Reducing Antioxidant Power
- GOD-POD: Glucose Oxidase-Peroxidase
- MIN-6: Mouse Insulinoma-6 (Pancreatic â-cells)
- NCCS: National Centre for Cell Science
- NCS: Newborn Calf Serum
- PAU: Punjab Agricultural University
- RE: Rutin Equivalent
- SPSS: Statistical Package for the Social Sciences
- TE: Trolox Equivalent

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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