

# Metabolic and anti-inflammatory effects of *Gymnema sylvestre* leaf extract in high-fat diet/streptozotocin- induced type 2 diabetic rats: evidence from metabolic profiling, gene expression, and network pharmacology

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## Research Article

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# Abstract

## Purpose

Obesity and diabetes-induced metabolic dysfunctions are growing global concerns and are often linked to dysfunction of fatty acid metabolism. *Gymnema sylvestre* is a traditionally used medicinal plant in India and touted to be beneficial for such conditions. However, its impact on lipid metabolism-related gene expression and chronic adipose tissue inflammation remains unexplored. This study aims to investigate effects of *G. sylvestre* leaf extract on lipid metabolism-related gene expression and chronic adipose tissue inflammation in a rat model of type 2 diabetes mellitus (T2DM).

## Methods

Obesity and adipose tissue dysfunction were induced by feeding high-fat diet (HFD) for 4 months in Wistar rats. This was followed by induction of pancreatic  $\beta$ -cell damage and T2DM in the rats by single intraperitoneal injection of Streptozotocin (STZ). The therapeutic efficacy of *G. sylvestre* leaf extract in regulating lipid metabolism-related gene expression, markers of chronic adipose tissue inflammation, and lipid metabolism was assessed and statistical analysis was done for analyzing groups.

## Results

*G. sylvestre* leaf extract exhibited anti-hyperglycemic and anti-hypertriglyceridemic effects. The extract significantly downregulated expression of lipogenic genes, viz., ELOVL6 and CCL2. Treatment with *G. sylvestre* leaf extract also reduced markers of chronic adipose tissue inflammation such as inflammatory cytokines (TNF $\alpha$  and IL6) and anthropometrical parameters (body mass index), besides regulating thyroid function and lipid profile.

## Conclusions

The findings of study provide scientific evidence that *G. sylvestre* leaf extract is a promising natural remedy as well as serve as potential therapeutic intervention for amelioration of adipose tissue dysfunction and related metabolic disorders in obese T2DM rats.

## Introduction

Obesity is an ever-growing pandemic and an escalating global health challenge. As per the World Health Organization and American Diabetes Association, by 2030, obesity is expected to affect 87 million and 439 million people in India and the world, respectively [1]. Despite ongoing research on diabetes and obesity-linked metabolic disorders and novel therapeutic alternatives being introduced, no single drug therapy has been found to effectively address all the components of metabolic syndrome. Available

drugs like metformin, ACE inhibitors target specific aspects of the metabolic syndrome, but they do not address it in totality [2]. Therapeutic interventions currently available for the treatment and management of obesity and adipose tissue dysfunction are limited and not accessible to all in less developed countries among the socio-economically poor sections of society.

Given the side effects of the commonly used therapeutic drugs, natural compounds such as *Gymnema sylvestre* offer potential alternatives [3,4,5]. To expand the repertoire of drugs, we have tested the effectiveness of *Gymnema sylvestre* leaf extract in the current study. The leaves of *Gymnema sylvestre*, commonly known as “Gurmar,” were used. The botanical name was checked and verified with <http://www.plantsoftheworldonline.org> (Accessed on 9 March, 2026). This study aims to investigate the impact of the extract on adipose tissue dysfunction, lipid metabolism, and inflammatory markers in a T2DM rat model.

The herbal extract rich in triterpenoid saponin glycosides and gymnemic acids (GA) is obtained from leaves of *G. sylvestre*, family Asclepediaceae, typically found in tropical forests of southern and central India, China, tropical Africa, and Australia. Although *G. sylvestre* has been traditionally used for diabetes management in Ayurveda, its molecular effects on lipid metabolism and inflammatory genes remain largely unexplored. The aim of this study is to find out the possible pathways involved, for which we have studied the expression of different genes associated with metabolic disorders, including those related to fatty acid metabolism. To our knowledge, few previous studies have examined the glycemic effects of *G. sylvestre*. To our knowledge, this is among the first studies evaluating the therapeutic efficacy of a standardized *G. sylvestre* extract in the treatment of T2DM in a rat model. Further, this study evaluates the effects of this extract on the crosstalk between lipid metabolism, adipose-associated inflammation, adipokine regulation, and metabolic dysfunction in a diet/STZ model that remains insufficiently explored.

## Materials and Methods

### In Vivo Study

**Place of study:** The study was conducted at ICAR-IVRI, Bareilly, India and manuscript work at ICAR-NRCM, Nagaland, India. (Detailed methodology of in-vivo studies provided in Online resource 1).

**Chemicals and kits:** Streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) was prepared in 100 mM citrate buffer (pH 4.5). Analytical kits were obtained from Coral Clinical Systems, Goa, India. Leptin, Adiponectin, and Insulin ELISA kits were procured from Invitrogen, Thermo Fisher Scientific (Waltham, MA, USA). All analyses were done according to the manufacturer’s instructions.

### Location of experiment

The experiment was conducted at ICAR-IVRI, Bareilly, India, with prior permission from the Institute Animal Ethics Committee. The study was conducted and reported in accordance with proper guidelines to promote transparent, reproducible, and rigorous reporting of animal research.

**Experimental Design:** The study was conducted using 40 male Wistar rats (IVRI2CQ strain, 16 weeks, 280–290g), acclimatized 7 days, then divided into 5 groups (n=8): standard diet (N), HFD (A), HFD+STZ (B), insulin-treated (C), extract-treated (D) (Details provided in Online resource 2: Table 2). Rats were individually housed; body weight, food/water intake monitored daily. Formulation and standardisation were done by the Division of Nutrition at the Institute of ICAR-IVRI, Bareilly.

**Extract Preparation:** Fresh *G. sylvestre* leaves (collected from ICAR-IVRI, Izatnagar, Bareilly, Northern India during February-March) were authenticated by the Botanical Survey of India (Voucher No. CNH/1-1/2020 Tech 14) and validated taxonomically. A hydro-ethanolic extract was prepared from dried, powdered leaves. The following formula was used for percentage yield of extract [6].

Extraction yield (%) = [weight of dried extract / weight of starting plant material] × 100.

The percentage yield of ethanol extract was found to be 10.7% (w/w). The EC<sub>50</sub> was determined using 40 obese male Wistar rats (280–290g weight) across 5 dose groups (100, 150, 200, 250, 300 mg/kg) following initial oral dextrose administration (2 mg/kg). The dose that reduced blood glucose by 50% within 1–2 hrs was identified as EC<sub>50</sub>. This extract (EC<sub>50</sub>) was administered orally for 90 days via gastric lavage. Prior to this, the extract underwent quality control at the Quality Control laboratory of ICAR-IVRI, Bareilly.

**Obesity/Diabetes Induction:** Rats were fed a high-fat diet (HFD: 58% fat, 25% protein, 17% carbohydrate) for 4 months to induce insulin resistance, followed by STZ injection per established protocol [7] (Details in Online resource 2: Table 2). Obesity was confirmed via BMI (normal: 0.45–0.68 g/cm<sup>2</sup> [8]; obese: >0.68 g/cm<sup>2</sup> [9] after 16 weeks; all experimental rats successfully attained the target BMI. STZ (35 mg/kg, IP, in citrate buffer) was administered after a 12-hr fast to induce β-cell dysfunction [10] and T2DM-like pathology (hyperglycemia, glycosuria, dyslipidemia, ketonemia) [11]. Controls received only citrate buffer intraperitoneally.

**Determination of Diabetes:** Diabetic rats showed polyuria, polyphagia, and polydipsia. Diabetes onset was defined as plasma glucose ≥300 mg/dl 72 hrs post-STZ. Insulin resistance was calculated with formula: HOMA-R = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5.

**Efficacy Evaluation of *G. sylvestre* Leaf Extract:** BMI was measured weekly [12] and urinalysis was assessed for glucose (Benedict's reagent) and ketones (Ornialysis® test strips). Blood analysis was conducted on Days 0, 45, 90 for plasma glucose, haematological parameters and serum for lipid profile (triglycerides, total cholesterol, LDL-C, HDL-C, VLDL-C), and thyroid panel (T3, T4, TSH) [13]. qPCR analysis was done with gene-specific primer sets (SSO fast EvaGreen Supermix, Bio-Rad) (Primer list provided in Online resource 2: Table 3) and 18sRNA gene was used as housekeeping control [14].

## In Silico Studies

**Data Collection:** Phytochemicals from *G. sylvestre* leaf extract with antidiabetic activity (Gymnemagenin, Gymnemic acids I/IV/VII, Lupeol, Quercitol, Stigmasterol) were identified via IMPPAT 2.0. Targets were predicted using SuperPred and Swiss Target Prediction; diabetes-related targets (Relevance score  $\geq 1$ ) were obtained from GeneCards. A Venn diagram tool identified shared targets between the extract and diabetes. (Detailed methodology of in-silico studies provided in Online resource 1).

**PPI Network:** Core targets were input into STRING to build a PPI network [15], visualized in Cytoscape (v3.8.0) [16]. Top 10 hub genes were ranked by node degree using the CytoHubba plug-in [17].

**GO/KEGG Analysis:** ShinyGO 0.82 was used for GO functional enrichment (biological processes, cellular components, molecular functions; higher counts = stronger association) and KEGG pathway enrichment (visualized via bubble diagrams/histograms; significance at  $p < 0.05$ ).

**Molecular Docking:** AutoDock Tools v1.5.7 assessed binding affinity between phytochemicals and chemokine receptors CCR2, CXCR4, CXCR7, and CCR5 (structures from RCSB PDB). Ligands (Gymnemagenin, Lupeol, Stigmasterol; from PubChem, SDF converted to PDB via OpenBabelGUI) were docked as follows: Gymnemagenin– CXCR4/CXCR7/CCR2/CCR5; Lupeol–CXCR4/CCR2/CCR5; Stigmasterol–CXCR7/CCR2/CCR5 (threshold  $\leq -5.0$  kcal/mol). Complexes were visualized using BIOVIA Discovery Studio (2D) and UCSF ChimeraX (3D).

**Statistical analysis:** SPSS v22 was used for statistical (SPSS Inc., Chicago, USA). The parametric data was reported as Mean  $\pm$  SE, non-parametric data as Score means  $\pm$  SE. Two-way ANOVA (General Linear Model) with Tukey's post-hoc test was applied [18] and significance was set at  $p < 0.05$ . For biochemical parameters measured at Days 0, 45, 90, two-way ANOVA with treatment group, time and group  $\times$  time interaction.

## Results

### In Vivo Study

#### HFD and STZ are responsible for the upregulation of the expression of genes involved in lipid metabolism

The data show that there was no significant difference in ELOVL6 expression between rats in HFD alone versus HFD+STZ (diabetic) groups (Fig. 1A). The study also revealed an upregulation of CCL2 gene, having more pronounced effect in HFD group followed by HFD+STZ group (Fig. 1C). Additionally, upregulation of the IL6 gene was observed in both HFD and HFD+STZ group (Fig. 1E). The current data also showed that expression of TNF $\alpha$  was markedly increased in both HFD+STZ rats and HFD group compared with normal control rats ( $p < 0.05$ ) (Fig. 1G).

#### *G. sylvestre* leaf extract downregulates the expression of lipid metabolism-related genes

**G. sylvestre leaf extract downregulates expression of the ELOVL6 gene**

The ELOVL6 expression gets perturbed as expressed earlier in T2DM rat model. The extract group had a significantly lower expression level of ELOVL6 than insulin-treated group on day 45 and day 90 (Fig. 1B) whereas insulin group showed an increase in its expression. To the best of our knowledge, this is the first study demonstrating the anti-lipidemic action of *G. sylvestre* leaf extract at genetic level via regulation of ELOVL6.

**G. sylvestre leaf extract downregulates expression of the CCL2 gene**

The effect of *G. sylvestre* leaf extract on MCP-1/CCL2 has not been scientifically documented. Therefore, the data showed that both the extract and insulin treatment downregulated the expression of the inflammatory cytokine CCL2 gene, but the extent of downregulation was more with *G. sylvestre* leaf extract in T2DM rat model (Fig. 1D).

**G. sylvestre leaf extract downregulate the expression of markers of chronic adipose tissue inflammation**

Expression of IL6 was found to be significantly elevated in both HFD group and HFD+STZ groups, compared to healthy rats. In contrast, treatments with *G. sylvestre* were found to downregulate expression of IL6 (Fig. 1F) as well as pro-inflammatory cytokine TNF $\alpha$  expression. Fig. 3H demonstrates that TNF $\alpha$  gene expression was downregulated at 90 day in *G. sylvestre* leaf extract-treated group compared to insulin-treated group.

**G. sylvestre leaf extract treatment improved anthropometrical parameters and markers of obesity in T2DM rat model**

**Body mass index (BMI)**

The data of BMI is presented in Table 1. Baseline BMI values were statistically similar across all groups. BMI declined significantly ( $p<0.05$ ) in all diabetic treatment groups compared to the non-diabetic HFD group, with the insulin-treated group experiencing the greatest weight and body condition loss. By Day 90, insulin-treated rats had lower BMI than untreated diabetic controls, though not statistically significant. In contrast, *G. sylvestre* leaf extract restored body weight close to baseline values, unlike the marked deterioration observed with insulin treatment.

**Table 1.** *Gymnema sylvestre* leaf extract improves body mass index values (g/cm<sup>2</sup>) in high-fat diet and streptozotocin-induced type 2 diabetic rats

| Groups                            | Day 0                     | Day 45       | Day 90                    |
|-----------------------------------|---------------------------|--------------|---------------------------|
| High Fat Diet (HFD) fed group     | 0.740± 0.005              | 0.776± 0.005 | 0.796± 0.005              |
| Diabetic control (HFD+ STZ) group | 0.728± 0.005 <sup>#</sup> | 0.686± 0.005 | 0.666± 0.005 <sup>#</sup> |
| Insulin treated group             | 0.730± 0.005 <sup>+</sup> | 0.673± 0.005 | 0.643± 0.005 <sup>+</sup> |
| Extract treated group             | 0.731± 0.005              | 0.686± 0.005 | 0.723± 0.005              |

Note: Values are expressed as mean ± SEM (n = 8). <sup>#</sup>*p* < 0.05, <sup>+</sup>*p* < 0.05

## Effects of *G. sylvestre* leaf extract on urine glucose and urine ketone bodies in T2DM rats

### Urine glucose

Baseline urine glucose levels were statistically similar across all diabetic groups (*p*>0.05), while HFD-fed rats had negligible amounts (Fig. 2A). Levels remained within same range across HFD+STZ, insulin-treated, and extract-treated groups through Day 45; however, a significant reduction (*p*<0.001) was observed exclusively in the extract-treated group by Day 90.

### Urine ketone bodies

Baseline urine ketone levels were comparable across all diabetic groups (*p*>0.05), with negligible amounts in the HFD group. While all groups showed similar ketone levels at Day 45, the extract-treated group demonstrated a progressive and significant decrease (*p*<0.001) by Days 45 and 90, reaching values statistically similar to the HFD group by Day 90 (*p*>0.05), indicating comparable beneficial effects to insulin in ameliorating ketonuria in the T2DM rat model (Fig. 2B).

## *G. sylvestre* leaf extract treatment improved blood biochemical parameters of T2DM rats

### Plasma Glucose (PG)

Prior to STZ injection, the mean plasma glucose levels in the rats ranged from 88.45-111.67 mg/dL. After 72 hours of injection, mean glucose levels increased to higher values, ranging from 397.52-457.80 mg/dL in diabetic rats of all groups. The insulin-treated group had plasma glucose of 79.06±1.66 mg/dL, and value was statistically similar to extract group (*p*> 0.05) on day 45 (Fig. 2C). On day 90, plasma glucose level of insulin-treated group (64.66±1.92 mg/dL) was significantly lower than extract-treated group (86.16±2.10 mg/dL). The results indicate that *G. sylvestre* leaf extract has a potent anti-hyperglycemic activity that was not statistically different from insulin-treated animals.

### Serum Total Cholesterol (TC)

Total cholesterol in rats (presented as Mean ± SE mg/dL) in extract-treated and insulin groups was in the range of 181.46±1.65 to 95.83±1.51 and 180.71±1.42 to 128.78 ±3.30 mg/dL, respectively which were

statistically similar ( $p>0.05$ ) (Fig. 2D). Thus, *G. sylvestre* leaf extract has potent anti-hypercholesterolemic properties that were not statistically different from insulin-treated animals.

### **Serum Triglycerides (TGs)**

HFD+STZ group had significantly higher ( $p<0.001$ ) serum TG levels ranging from  $181.89\pm1.64$  to  $310.10\pm7.11$  mg/dL than treatment groups throughout study. The values of TGs in insulin-treated rats ranged from  $183.13\pm1.38$  to  $104.24\pm3.02$  mg/dL, and expectedly, the value was significantly lower than HFD+STZ group on 90<sup>th</sup> day and were statistically similar ( $p>0.05$ ) in extract-treated rats (Fig. 2E). Thus, the extract has comparable anti-hypertriglyceridemic property as that of insulin.

### **High-density lipoprotein cholesterol (HDL-C)**

During this experiment, it was found that Mean  $\pm$  SE values of serum HDL-C level of HFD+STZ group had significantly lower ( $p<0.001$ ) values ( $30.30\pm1.16$  to  $9.30\pm0.59$  mg/dL) than treated groups. The group treated with insulin had significantly higher ( $p<0.001$ ) serum HDL-C levels ( $27.83\pm1.66$  to  $34.37\pm1.24$  mg/dL) than HFD+STZ group. These values were statistically similar to extract-treated group ( $p>0.05$ ) (Fig. 2F). The results indicate that *G. sylvestre* leaf extract is associated with a more favourable lipid profile and has the potency to increase serum HDL-C levels.

### **Low-density lipoprotein cholesterol (LDL-C)**

The values of serum LDL-C level of rats in HFD+STZ group were significantly higher ( $p<0.001$ ) than insulin and extract-treated groups. The serum LDL-C of extract-treated group were significantly lower ( $p<0.001$ ) than HFD+STZ group (Fig. 2G). The results indicate that extract has potency to decrease serum LDL-C levels.

### **Very-low-density lipoprotein cholesterol (VLDL-C)**

Mean $\pm$ SE values of serum VLDL-C level in HFD+STZ group ranged from  $36.38\pm0.33$  to  $62.02\pm1.42$  mg/dL, and was significantly higher ( $p<0.001$ ) than treated groups. The serum VLDL-C of extract-treated rats were significantly lower ( $p<0.001$ ) than HFD+STZ group (Fig. 2H). Therefore, *G. sylvestre* leaf extract can decrease serum VLDL-C levels in T2DM rats.

### **Thyroid profile was improved by *G. sylvestre* leaf extract treatment in T2DM model rats**

#### **Tetraiodothyronine or Thyroxine (T<sub>4</sub>)**

The mean values of serum T<sub>4</sub> in rats treated with extract group were statistically similar to the values in HFD+STZ and insulin-treated groups (Fig. 3A). The serum T<sub>4</sub> levels under insulin-treated group ranged from  $4.88\pm0.15$  to  $5.16\pm0.06$  ng/dL. The results indicate that *G. sylvestre* leaf extract has comparable effect on serum T<sub>4</sub> levels as that of insulin therapy.

#### **Triiodothyronine (T<sub>3</sub>)**

The Mean±SE values of serum T3 levels of HFD+STZ group were statistically similar to treated groups. The values of serum T3 in extract-treated group were statistically similar (p>0.05) to insulin-treated group. The insulin group had serum T3 levels ranging from 24.10±1.29 to 68.40±12.95 ng/dL (Fig. 3B). The results indicate that *G. sylvestre* leaf extract has comparable effect on the serum T3 levels as that of insulin therapy alone.

Thyroid Stimulating Hormone (TSH)

The serum TSH levels of HFD+STZ group ranged from 0.76±0.08 to 2.35±1.59 mIU/L. The mean values in extract-treated group did not show statistically significant difference compared to HFD+STZ group. The insulin-treated group had serum TSH levels ranging from 0.86±0.13 to 1.86±0.08 mIU/L, and mean value was statistically similar to extract group (p>0.05). Therefore, results indicate that *G. sylvestre* leaf extract does not affect serum TSH levels (Fig. 3C).

Adipokine secretion was recouped by *G. sylvestre* leaf extract treatment in obese rats with T2DM

Serum Leptin

No significant intergroup differences were observed in leptin levels (p>0.05), though HFD vs. insulin-treated (p=0.0575) and HFD vs. standard diet (p=0.0766) approached borderline significance, suggesting these interventions may help regulate leptin levels (Table 2). At Day 0, extract- and insulin-treated groups showed the highest leptin levels. By Day 45, slight reductions were noted in HFD and HFD+STZ groups, while both treatment groups remained elevated. By Day 90, leptin dropped significantly in HFD and HFD+STZ groups, whereas insulin- and extract-treated groups maintained moderate levels (Fig. 3D).

**Table 2.** Effect of Insulin and *Gymnema sylvestre* leaf extract on serum leptin levels (pg/mL) (Mean ± SEM) in high-fat diet and Streptozotocin-induced Type 2 diabetic rats

| Groups                                                  | Day 0           | Day 45         | Day 90         |
|---------------------------------------------------------|-----------------|----------------|----------------|
| Standard Diet (Group N)                                 | 202.00 ± 109.50 | 232.70 ± 120   | 222.20 ± 94.50 |
| HFD (Group A)                                           | 105.64 ± 28.81  | 80.60 ± 12     | 69.55 ± 7.67   |
| Diabetic Control/ HFD+STZ (Group B)                     | 257.49 ± 197.03 | 160.50 ± 80    | 68.14 ± 65.06  |
| Insulin-Treated (Group C)                               | 269.00 ± 112.11 | 229.00 ± 105.1 | 183.55 ± 81.43 |
| <i>Gymnema sylvestre</i> leaf extract-treated (Group D) | 270.00 ± 134.7  | 194.00 ± 85.76 | 156.05 ± 75.53 |

Note: Values are expressed as mean ± SEM (n = 8).

Serum Adiponectin

Both “Group” (p=0.0007) and “Day” (p=0.0419) significantly influenced serum adiponectin levels. HFD differed significantly from standard diet (p=0.0294), and HFD+STZ differed from insulin-treated (p=0.0111) and standard diet (p=0.0020) groups, while extract-treated group showed no significant difference from others (Fig. 3E). Baseline adiponectin levels were similar across all groups (Table 3). By Day 45, levels declined in the three diabetic groups compared to HFD and standard diet groups, with HFD+STZ showing further decrease compared to HFD (p<0.001). No significant difference was observed between insulin- and extract-treated groups. Leptin correlated with fasting insulin (r=0.420, p<0.05) and glucose (r=-0.415, p<0.05), while adiponectin correlated with glucose (r=-0.359, p<0.05) and leptin (r=0.530, p<0.01).

**Table 3.** Effect of Insulin and *Gymnema sylvestre* leaf extract on adiponectin levels (ng/mL) (Mean ± SEM) in the serum of high-fat diet and Streptozotocin-induced Type 2 diabetic rats

| Groups                                                  | Day 0          | Day 45                      | Day 90                     |
|---------------------------------------------------------|----------------|-----------------------------|----------------------------|
| Standard Diet (Group N)                                 | 120.22 ± 52.50 | 114.71 ± 58.10              | 109.20 ± 85.52             |
| HFD (Group A)                                           | 95.64 ± 58.31  | 54.10±33.55 <sup>##</sup>   | 12.55 ± 7.67 <sup>##</sup> |
| Diabetic Control/ HFD+STZ (Group B)                     | 28.49 ± 11.03  | 23.81 ± 10.12 <sup>##</sup> | 19.14±11.06 <sup>##</sup>  |
| Insulin-Treated (Group C)                               | 109.12±38.50   | 90.00 ± 39.57               | 86.00 ± 35.37              |
| <i>Gymnema sylvestre</i> leaf extract-Treated (Group D) | 81.89 ± 27.33  | 63.02 ± 29.56               | 58.21 ± 29.51              |

Note: Values are expressed as mean ± SEM (n = 8). <sup>###</sup>p < 0.001

### Serum Adiponectin/Leptin ratio (A/L Ratio)

A/L ratio trends over 90 days varied considerably across groups (Table 4, Fig. 3F). The HFD group showed substantial decline, decreasing 25.86% (671.22) by Day 45 and 80.07% (180.45) by Day 90, suggesting metabolic imbalance. The HFD+STZ group displayed notable increases of 34.07% (148.35) on Days 45 and 153.85% (280.89) on day 90, reflecting progressive metabolic imbalance. The insulin-treated group showed a minor decline of 3.12% (393.01) at Day 45 followed by a moderate increase of 15.50% (468.54) by Day 90. The extract-treated group demonstrated consistent improvement, rising 7.11% (324.85) by Day 45 and 22.99% (373.02) by Day 90, suggesting a progressive therapeutic benefit. Overall, *G. sylvestre* extract appeared particularly promising in stabilizing and improving the A/L ratio over time.

**Table 4.** Effect of Insulin and *Gymnema sylvestre* leaf extract on A/L Ratio (Means) in the serum of High-fat Diet and Streptozotocin-induced Type 2 diabetic rats (n=8)

| Groups                                            | Day 0  | Day 45 | Day 90 |
|---------------------------------------------------|--------|--------|--------|
| Standard Diet (Group N)                           | 595.15 | 492.95 | 491.45 |
| High-Fat Diet (HFD) (Group A)                     | 905.34 | 671.22 | 180.45 |
| Diabetic Control/ HFD+STZ (Group B)               | 110.65 | 148.35 | 280.89 |
| Insulin-Treated (Group C)                         | 405.65 | 393.01 | 468.54 |
| Gymnema Sylvestre leaf extract- Treated (Group D) | 303.30 | 324.85 | 373.02 |

### Identification of anti-diabetic targets of *G. sylvestre* leaf extract

Venn diagram analysis of *G. sylvestre* leaf extract targets and diabetes-related therapeutic targets (Fig. 4A) identified 494 intersectional anti-diabetic targets.

### In Silico Studies

#### PPI construction

The 494 core targets were integrated into the STRING platform for *Rattus norvegicus* to generate a PPI network map comprising 491 nodes and 781 edges (Fig. 4B). Cytoscape analysis using the degree algorithm identified 10 hub genes/key targets: Prkaca, Src, Pik3r1, Pik3cd, Pik3cb, Pik3ca, Mapk3, Mapk1, Ptpn11, and Jak2 (Fig. 4C).

#### GO and KEGG Enrichment Analysis

ShinyGO analysis of 10 identified targets with statistically significant ( $p < 0.05$ ) biological processes, molecular functions, and cellular components. KEGG enrichment analysis further revealed 15 significant pathways ( $p < 0.05$ ) associated with anti-diabetic mechanisms (Fig. 4D-G).

#### Molecular docking

The details of the molecular docking of different phytochemicals and their core targets are given in Table 5.

Table 5. Molecular docking results of different phytochemicals and core targets

| Sl. No. | Phytochemical | Target | Affinity (kJ·mol <sup>-1</sup> ) | Interacting residues                                                                       |
|---------|---------------|--------|----------------------------------|--------------------------------------------------------------------------------------------|
| 1.      | Gymnemagenin  | CXCR4  | -7.45                            | ARG152, CYS75, LEU76, LYS74, LYS150                                                        |
|         |               | CXCR7  | -8.58                            | CYS196, TRP100, TYR51, LEU104, ARG197, ASN108, SER103                                      |
|         |               | CCR2   | -6.47                            | LYS150, ARG152, CYS75, LEU76, LYS74                                                        |
|         |               | CCR5   | -7.45                            | ILE296, ILE295, PHE299, THR152, THR148, LEU205, PHE117, ILE120, LEU121, ILE124             |
| 2.      | Lupeol        | CXCR4  | -11.28                           | ILE213, ILE209, PHE249, LEU246, ILE245, ILE253                                             |
|         |               | CCR2   | -8.97                            | LEU76, ARG152, LEU140, PHE147, ALA151, PHE156, ALA148, ILE136                              |
|         |               | CCR5   | -8.17                            | ILE54, LEU308, TYR307, PHE311, ALA298, LEU50, ILE151, VAL46, PHE43, ILE295, PRO294         |
| 3.      | Stigmasterol  | CXCR7  | -9.16                            | LEU104, TRP110, TRP100, HIS121, TYR268, PHE124, LEU305                                     |
|         |               | CCR2   | -9.63                            | ALA241, VAL244, ARG237, LYS240, LYS311, TYR315, PHE312, LEU67, ILE66, VAL63, TYR305, LEU81 |
|         |               | CCR5   | -9.48                            | VAL147, ALA298, PHE304, ILE54, LEU50, VAL150, VAL51, VAL154, VAL46, PRO294, PHE43          |

Gymnemagenin displayed moderate binding affinities across the tested receptors. Interaction with CCR2 was weaker ( $-6.47 \text{ kcal}\cdot\text{mol}^{-1}$ ). (Online resource 3. Fig. 1A and B). In CCR5, Gymnemagenin showed similar affinity as with CXCR4 ( $-7.45 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Online resource 3. Fig. 1C and D). Against CXCR4, it showed an affinity of  $-7.45 \text{ kcal}\cdot\text{mol}^{-1}$ , (Online resource 3. Fig. 1E and F). The binding was comparatively stronger with CXCR7 ( $-8.58 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Online resource 3. Fig. 1G and H).

Lupeol exhibited stronger binding affinities compared to Gymnemagenin. Lupeol demonstrated notable binding with CCR2 ( $-8.97 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Online resource 3. Fig. 2A and B). The highest affinity was observed for CXCR4 ( $-11.28 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Online resource 3. Fig. 2C and D). For CCR5, the ligand showed an affinity of  $-8.17 \text{ kcal}\cdot\text{mol}^{-1}$  (Online resource 3. Fig. 2E and F).

Stigmasterol showed the most consistent and strong binding among the compounds, particularly with CXCR7, CCR2, and CCR5. In CCR2, Stigmasterol showed the highest affinity of the group ( $-9.63 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Fig. 5A and B). followed by CCR5 ( $-9.48 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Fig. 5C and D) and CXCR7 ( $-9.16 \text{ kcal}\cdot\text{mol}^{-1}$ ) (Fig. 5E and F).

# Discussion

## Metabolic effects of *G. sylvestre* extract

### Effects on Blood biochemical parameters

Plasma glucose increased progressively in the HFD group, while *G. sylvestre* extract maintained glucose within the normal range. In contrast, the insulin-treated group frequently became hypoglycemic, requiring dose adjustments in some insulin-treated rats [19], inferring that the extract is a potent anti-hyperglycemic agent and found to have better control on plasma glycemic control than insulin.

HFD followed by STZ induced glycol-metabolic changes manifested by levels of plasma glucose, lipid profile, and thyroid profile in the diabetic rats, with highly significant ( $p < 0.001$ ) elevations in serum TG, TC, LDL-C, and VLDL-C [20].

Persistent hyperglycemia throughout the experiment confirmed reduced insulin action in peripheral tissues, indicative of insulin resistance — a common denominator across most metabolic syndromes including T2DM. The extract gradually and stably reduced elevated serum TG, TC, VLDL-C, and LDL-C in hyperlipidemic rats. It was also observed that decreased values of the anti-atherogenic index (AAI) as well as serum HDL-C were also reversed towards normalization after the extract treatment implying that it has anti-atherosclerotic potential. Given the well-established link between elevated TC and atherosclerosis, the extract's ability to lower TC through reduction of VLDL-C and LDL-C alongside increased HDL-C may collectively reduce the risk of congestive heart disease. The extract normalised serum cholesterol concentrations, demonstrating robust hypolipidemic activity comparable with insulin therapy.

### Effects on Thyroid profile

The thyroid profile consisting of measurement of  $T_3$ ,  $T_4$ , and TSH concentration in the serum revealed that there was no significant change in these levels after administration of the extract orally. As no significant changes in thyroid hormone concentrations were observed by the administration of the extract in HFD+STZ-induced T2DM rats, it further appears that the effects of the tested material might not have been mediated through alterations of thyroid function. However, no such studies were found to compare our findings. So, this is the first study to report no direct action of *G. sylvestre* leaf extract on thyroid hormone levels.

## Effects of *G. sylvestre* extract on obesity and adipokines

### Effects on anthropometrical parameters and markers of obesity

BMI progressively increased in HFD-fed rats throughout the study [21, 22]. By Day 90, the extract-treated group showed body weights restored close to baseline, indicating anabolic effects, unlike the HFD group [23]. The HFD+STZ group lost weight due to protein and fat catabolism and polyphagia [19]. Urine

glucose and ketone bodies were elevated in the HFD+STZ group, whereas the values were reduced in the extract-treated group, consistent with *G. sylvestre*'s reported ability to improve glycemic control and attenuate diabetic ketoacidosis [24].

### **Effects on Adipokine secretion**

This study examined the effects of the extract on adiponectin and leptin levels in HFD+STZ-induced T2DM Wistar rats. Adiponectin, known for enhancing insulin sensitivity and exerting anti-inflammatory effects, was significantly suppressed in the HFD+STZ group, consistent with prior reports [25]. The extract-treated group significantly restored adiponectin levels over time, comparable to the insulin-treated group, indicating improved insulin sensitivity and metabolic homeostasis [26]. Leptin, secreted by adipose tissue to regulate appetite and energy balance, was decreased in the HFD+STZ group at later stages, reflecting leptin resistance in obesity-related diabetes [27]. The extract-treated group maintained relatively higher leptin levels, suggesting improved leptin regulation, while the A/L ratio trended toward metabolic recovery [28]. The adiponectin/leptin (A/L) ratio, an emerging biomarker of insulin sensitivity, was significantly lower in the HFD+STZ group than in controls [29, 30]. Treatment with *G. sylvestre* leaf extract progressively increased the A/L ratio, indicating improved insulin sensitivity and metabolic function, aligning with evidence that adiponectin enhancement and reduced leptin resistance improve glycemic control [31].

### **Effects of *G. sylvestre* extract on inflammatory pathways**

#### **Effects on Lipid metabolism-related gene expression**

Fatty acid elongases and desaturases regulate hepatic and body lipid metabolism [32]. ELOVL6, expressed in the liver, adipose tissue, brain, skin, and kidney [33], elongates 12–16 carbon fatty acid chains [34]. Both HFD and HFD+STZ groups showed significantly elevated ELOVL6 levels compared to healthy rats. Since STZ is known to downregulate ELOVL6 [35], continuous HFD feeding likely reached the biological regulatory limit, rendering additional STZ ineffective. As ELOVL6 expression alone is insufficient to validate a T2DM model, additional parameters were assessed.

MCP-1/CCL2, a cytokine mediating chronic inflammation in obesity [36], was significantly elevated in HFD and HFD+STZ groups at Day 0. Since STZ does not affect CCL2 in hepatic stellate cells [37], saturated fat-rich diets appear primarily responsible for adipose tissue inflammation. Similarly, pro-inflammatory markers IL6 and TNF $\alpha$  were elevated in the HFD group relative to healthy controls [38, 39]. STZ injection in hyperlipidemic rats reduced IL6 expression within 72 hours compared to HFD alone, though levels remained higher than in healthy controls. Given IL6's dual pro- and anti-inflammatory role [40], and the documented anti-inflammatory properties of *G. sylvestre* [41], our data evidence that *G. sylvestre* leaf extract ameliorates obesity-induced inflammation through downregulation of pro-inflammatory cytokines, reducing triglyceride accumulation and enhancing fatty acid metabolism [42]. Diet-induced obesity markedly activated adipose inflammatory signalling, whereas *Gymnema* treatment effectively reversed this response by suppressing IL6 and TNF- $\alpha$  expression.

## Network pharmacology and docking studies

T2DM involves insulin resistance,  $\beta$ -cell dysfunction, adipose inflammation, and dyslipidemia. This study combines in vivo experiments with network pharmacology to identify molecular targets of *Gymnema sylvestre* leaf extract in T2DM.

From 494 overlapping anti-diabetic targets, PPI network analysis identified ten hub genes: Prkaca, Src, Pik3r1, Pik3cd, Pik3cb, Pik3ca, Mapk3, Mapk1, Ptpn11, and Jak2. KEGG analysis revealed 15 enriched pathways linked to inflammation, hormone signalling, and metabolism, correlating with in vivo findings: downregulation of ELOVL6, CCL2, TNF- $\alpha$ , and IL-6, and improved glucose, lipid profile, BMI, and adiponectin/leptin (A/L) ratio.

CCR2, CXCR4, CXCR7, and CCR5 were selected for molecular docking based on network pharmacology analysis identifying them as key targets involved in the inflammatory and metabolic processes investigated in this study. These chemokine receptors regulate leukocyte recruitment, adipose tissue inflammation, immune-cell infiltration, and metabolic dysfunction associated with obesity and type 2 diabetes mellitus. CCR2/CCL2 signaling promotes monocyte/macrophage recruitment into adipose tissue and obesity-associated insulin resistance, whereas CCR5 contributes to chronic adipose inflammation and metabolic dysfunction. CXCR4 and CXCR7 mediate chemokine signalling, immune-cell trafficking, inflammatory responses, and adipose tissue biology [43, 44]. Their expression in metabolically and immunologically relevant tissues further supports their potential involvement in the effects observed in the present study. Molecular docking was therefore performed to evaluate the interactions of *Gymnema sylvestre* phytoconstituents—gymnemagenin, lupeol, and stigmasterol—with these receptors, providing mechanistic hypotheses regarding the observed modulation of inflammatory markers and metabolic parameters.

Three compounds were docked against chemokine receptors CXCR4, CXCR7, CCR2, and CCR5 which resulted in the following:

Gymnemagenin: moderate affinity (−6.47 to −8.58 kcal/mol), strongest with CXCR7 (via TRP100, TYR51, ASN108); recurring ARG152/LEU76 contacts suggest conserved receptor-family binding.

Lupeol: stronger binding, especially CXCR4 (−11.28 kcal/mol) [45] via hydrophobic residues ILE213, ILE209, PHE249; also notable affinity for CCR2 (−8.97) and CCR5 (−8.17), aligning with its well-documented anti-inflammatory effects. Previous docking studies showed phytocompound lupeol exhibits strong binding with aldose reductase and other diabetic target proteins, with better docking scores and interactions [46].

Stigmasterol: most consistent profile—CCR2 (−9.63), CCR5 (−9.48), CXCR7 (−9.16 kcal/mol)—via hydrophobic/aromatic residues (VAL147, PHE304, TRP100), supporting its documented anti-inflammatory, cholesterol-lowering [47, 48], and possibly chemokine-modulating properties, especially relevant given CCR2's role in inflammation and cancer metastasis [49].

**Chemokine Signalling:** CCL2 recruits monocytes to adipose tissue, promoting macrophage infiltration and impaired insulin signalling [50]. JAK2 mediates IL-6/TNF- $\alpha$  signalling; MAPK1/3 activate inflammatory gene expression via AP-1; SRC supports cytokine and oxidative stress responses. The extract downregulated CCL2, TNF- $\alpha$ , and IL-6, suggesting suppression of chemokine signalling and better insulin sensitivity and normalized adipokine levels.

**Endocrine Resistance:** Defects in estrogen, leptin, and insulin receptor signalling contributes to glucose intolerance, lipid metabolism [51, 52]. PIK3CA/B/D/R1 (Class I PI3K) and MAPK1/SRC regulate insulin and ERK/JNK pathways. The extract improved A/L ratio, BMI, and leptin stability—indicating reversal of endocrine resistance.

**Estrogen Signalling:** ER signalling supports insulin sensitivity and lipid regulation; its loss raises T2DM risk [53, 54]. MAPK1/SRC mediate non-genomic ER signalling, while PIK3CA links ER to PI3K-Akt glucose uptake. The extract enhanced adiponectin, normalized A/L ratio, and improved lipid profile, which may be consistent with restored estrogen-linked metabolic regulation. The network pharmacology analysis suggested potential involvement of PI3K/Akt, MAPK, chemokine, and endocrine-related signalling pathways. These findings generate mechanistic hypotheses that require validation in future studies.

**Growth Hormone/ Relaxin Signalling:** PRKACA (via cAMP) and MAPK1 mediate GH/relaxin signalling, regulating lipolysis and muscle preservation [55]. The extract maintained BMI without hypoglycemia or muscle wasting, unlike insulin treatment, indicating support of energy homeostasis [56].

MAPK1 stood out as a key integrator linking inflammation, metabolism, and proliferation across pathways, supporting a polypharmacology model where *G. sylvestre* compounds act on multiple components of disease networks rather than one target—paralleling mechanisms of metformin (AMPK activation, cytokine suppression) and pioglitazone (PPAR- $\gamma$  agonism). No overt adverse effects were observed during the study; however, dedicated toxicological studies are required to establish the safety profile of the extract. In a previous study [11], the extract also showed a high safety margin, with no hepatotoxicity or nephrotoxicity reported at therapeutic doses.

No treatment-related adverse effects or overt signs of toxicity were observed during the experimental period. However, because this study was not intended as a dedicated toxicological assessment, these findings should not be regarded as evidence of the long-term safety of *Gymnema sylvestre* extract. Existing preclinical and clinical studies suggest that *G. sylvestre* preparations are generally well tolerated, although gastrointestinal adverse effects and potential interactions with glucose-lowering medications have been reported. Consequently, comprehensive toxicological studies, including hematological, biochemical, histopathological, and dose-escalation evaluations, are warranted to establish the safety profile of standardized *G. sylvestre* extracts before clinical translation.

The investigation has several limitations that should be acknowledged. First, the study included a relatively small cohort of male rats, limiting the generalizability of the findings across sexes and species.

Second, although network pharmacology and molecular docking identified potential molecular targets, these predictions were not experimentally validated at the protein or signalling pathway levels. Third, further phytochemical characterization and quantitative standardization of the extract are required. Fourth, the 90-day study duration does not permit conclusions regarding long-term efficacy or safety. Finally, the clinical relevance of these findings remains to be confirmed through well-designed controlled human studies.

## Conclusion

The results of the present study have successfully established that *Gymnema sylvestre* leaf extract has potent ameliorative effects on adipose tissue dysfunction of T2DM obese rats. It is anti-hyperglycemic and anti-hypertriglyceridemic, and was associated with down-regulation of expression of lipid metabolism-related gene (ELOVL6) and inflammatory gene (CCL2), down-regulates markers of chronic adipose tissue inflammation like inflammatory cytokines (TNF $\alpha$ , & IL6) and anthropometrical parameters (body mass index) besides metabolic regulation of thyroid function and lipid profile. The docking suggests potential molecular interactions, including downregulation of pro-inflammatory genes (TNF- $\alpha$ , IL-6, CCL2), improved adipokine profiles (increased adiponectin, decreased leptin resistance), improved lipid and glucose parameters (decreased PG, LDL-C, TGs and increased HDL-C), better BMI and A/L ratios as indicators of improved metabolic homeostasis. Thus, the docking analysis identified potential interactions between selected phytochemicals (Gymnemagenin, Gymnemic acids I/IV/VII, Lupeol, Quercitol, Stigmasterol) and chemokine receptors (CCL2, TNF- $\alpha$ , and IL-6), providing hypotheses for future mechanistic investigations.

The results in this study have also provided scientific evidence of *G. sylvestre* leaf extract as a promising herbal remedy for adipose tissue dysfunction and metabolic disorders. The findings from this study suggest that *G. sylvestre* leaf extract effectively modulates adiponectin and leptin levels, leading to improved A/L ratios in type 2 diabetic obese Wistar rats. This indicates its potential role in alleviating insulin resistance and metabolic dysfunction associated with diabetes. Future studies should explore the underlying molecular mechanisms and evaluate long-term effects in clinical settings to establish *G. sylvestre* leaf extract as a viable therapeutic intervention for metabolic disorders.

## Abbreviations

|                    |                            |
|--------------------|----------------------------|
| A/L ratio          | Adiponectin/Leptin ratio   |
| HFD                | High fat diet              |
| STZ                | Streptozotocin             |
| <i>G.sylvestre</i> | <i>Gymnema sylvestre</i> , |
| ANOVA              | Analysis of variance       |

|                    |                                                                             |
|--------------------|-----------------------------------------------------------------------------|
| TNF $\alpha$       | Tumor necrosis factor alpha                                                 |
| IL                 | Interleukin                                                                 |
| T2DM               | Type 2 diabetes mellitus                                                    |
| EC <sub>50</sub>   | Half maximal effective concentration                                        |
| $^{\circ}\text{C}$ | Degree Celsius                                                              |
| mg/kg              | milligram per kilogram                                                      |
| %                  | Percentage                                                                  |
| mg/dL              | milligram per decilitre                                                     |
| ICAR-IVRI          | Indian Council of Agriculture Research-Indian Veterinary Research Institute |
| BMI                | Body mass index                                                             |
| LDL-C              | Low-density lipoprotein cholesterol                                         |
| HDL-C              | High-density lipoprotein cholesterol                                        |
| VLDL-C             | Very-low density lipoprotein cholesterol                                    |
| T <sub>3</sub>     | Triiodothyronine                                                            |
| T <sub>4</sub>     | Thyroxine or tetraiodothyronine                                             |
| TSH                | Thyroid stimulating hormone                                                 |
| ELISA              | Enzyme-linked immunosorbent assay                                           |
| qPCR               | quantitative polymerase chain reaction                                      |
| ELOVL6             | Elongation of very long chain fatty acids protein 6                         |
| CCl2               | Chemokine (C-C motif) ligand 2                                              |
| PG                 | Plasma glucose                                                              |
| TC                 | Serum total cholesterol                                                     |
| TGs                | Serum Triglycerides                                                         |
| AAI                | anti-atherogenic index                                                      |

# Declarations

## Authors' contribution

Plabita Goswami: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing – review and editing. Dhaval J Kamothi: Formal analysis, Methodology, Software, Writing – review and editing. Sedeneinu Suohu: Formal analysis, Writing – review and editing. Vinod Kumar Dubey: Formal analysis, Software, Writing – review and editing. Saurabh Gupta: Formal analysis, Methodology. Sujoy Kumar Dhara: Formal analysis, Supervision. Sahadeb Dey: Conceptualization, Methodology, Supervision.

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## Data availability

Not applicable.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Ethics approval

The study was conducted at ICAR-IVRI, Bareilly, India, with prior permission from the Institute Animal Ethics Committee. The approval with regard to Laboratory or experimental animals is from IAEC/CPCSEA [No. F. 26-1/ 2018- 19/ J.D. (R) dated 05.08.2019].

## Consent for publication

Not applicable

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## Figures

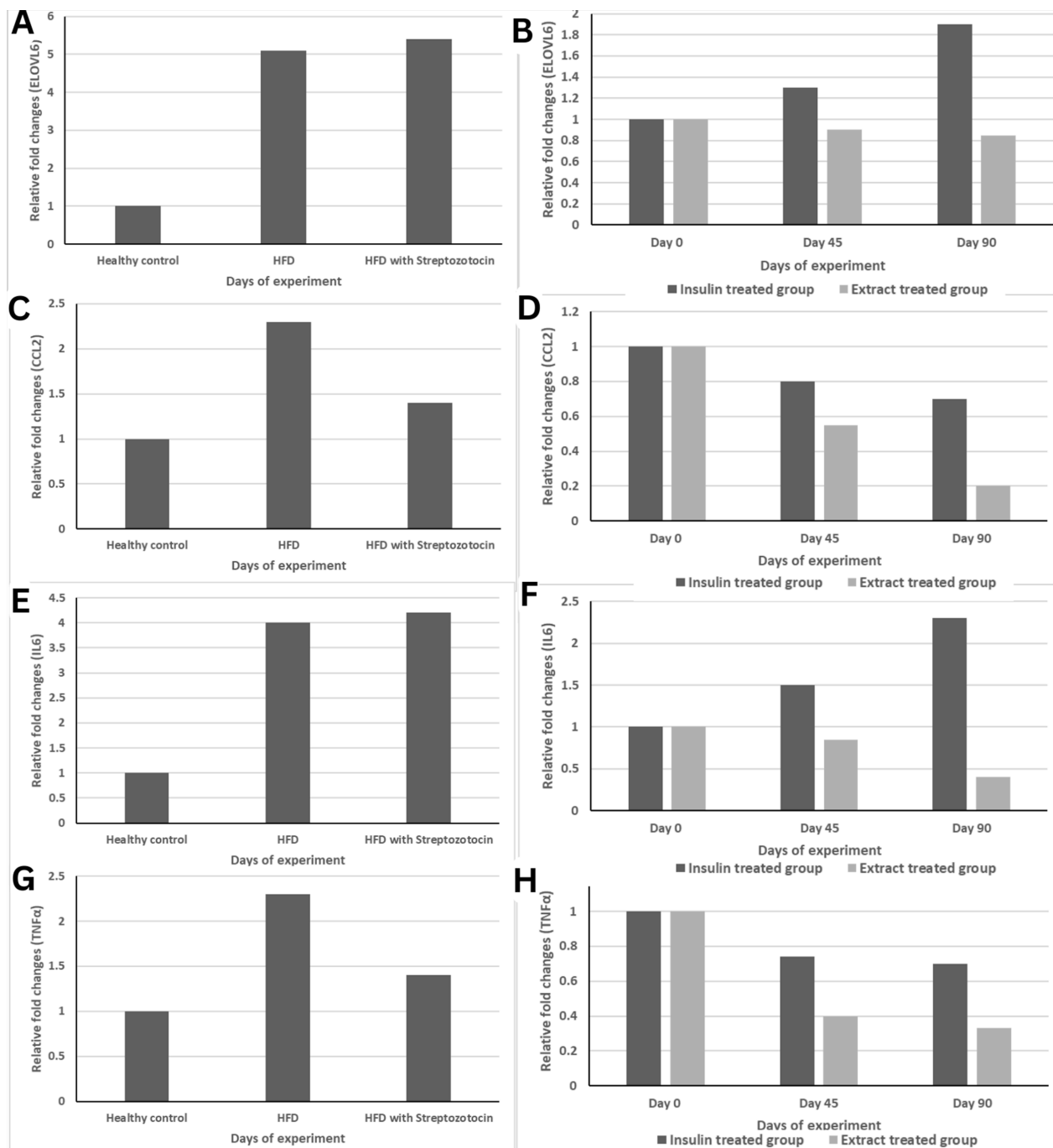


**Figure 1**

*Gymnema sylvestre* leaf extract treatment resulted in decreased expression of adipogenic and proinflammatory genes in T2DM rat model (n=8). Relative mRNA expression normalized to vehicle control group for the following qPCR analysis:

(A) Expression of ELOVL 6 gene on the 0<sup>th</sup> day of the study;

(B) Comparison was made between groups of rats treated with insulin vs *Gymnema sylvestre* leaf extract;

(C) Expression of CCL2 gene on the 0<sup>th</sup> day of the study;

- (D) Expression of CCL2 gene at 0, 45<sup>th</sup> & 90<sup>th</sup> day of the experiment (insulin vs *Gymnema sylvestre* leaf extract treated group)
- (E) Expression of IL-6 gene on the 0<sup>th</sup> day of the study;
- (F) Expression pattern of IL-6 gene on the 0<sup>th</sup>, 45<sup>th</sup> & 90<sup>th</sup> day with respect to insulin treated and *Gymnema sylvestre* leaf extract treated groups
- (G) Expression of TNF-a gene on the 0<sup>th</sup> day of the study;
- (H) Expression profiles of TNF-a gene in conventional insulin vs *Gymnema sylvestre* leaf extract treatment on the respective 0<sup>th</sup>, 45<sup>th</sup> & 90<sup>th</sup> day of the study.

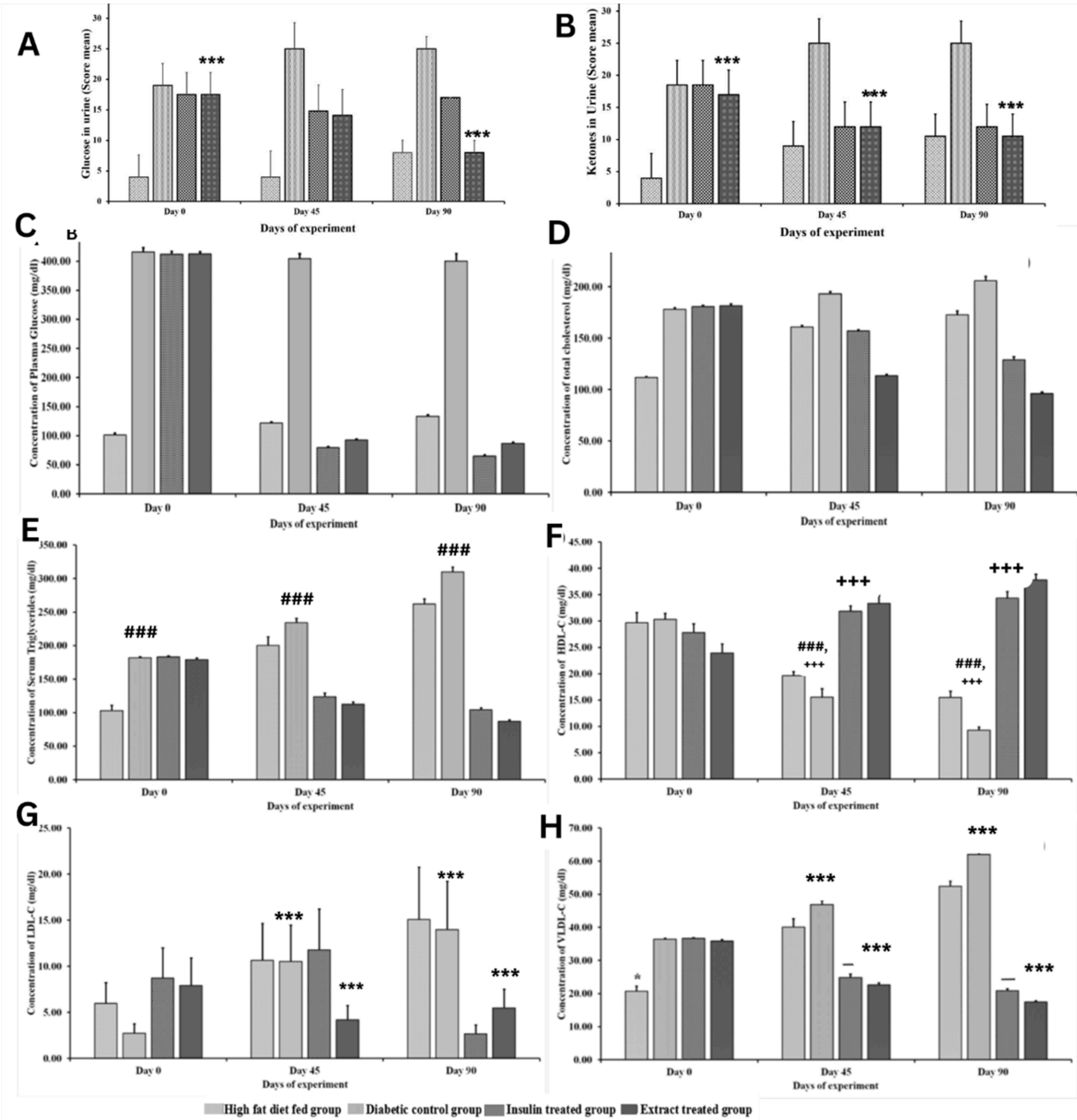


Figure 2

*Gymnema sylvestre* leaf extract treatment restores urine glucose and ketone levels in experimental rats (A-B). Data are presented as the mean  $\pm$  SD (n=8). \*\*\*  $p < 0.001$ . Treatment with *Gymnema sylvestre* leaf extract shows improvement of biochemical parameters in T2DM rat model (C) Plasma Glucose. (D) Serum Total Cholesterol. (E) Serum Triglycerides. (F) Serum High-density lipoprotein cholesterol (HDL-C). (G) Serum Low-density lipoprotein cholesterol (LDL-C). (H) Serum Very low-density lipoprotein cholesterol (VLDL-C. Data are presented as the mean  $\pm$  SD (n=8). ###  $p < 0.001$  (Comparison with diabetic control group), +++  $p < 0.001$  (Comparison with insulin treated group), \*\*\*  $p < 0.001$  (Comparison with extract group)

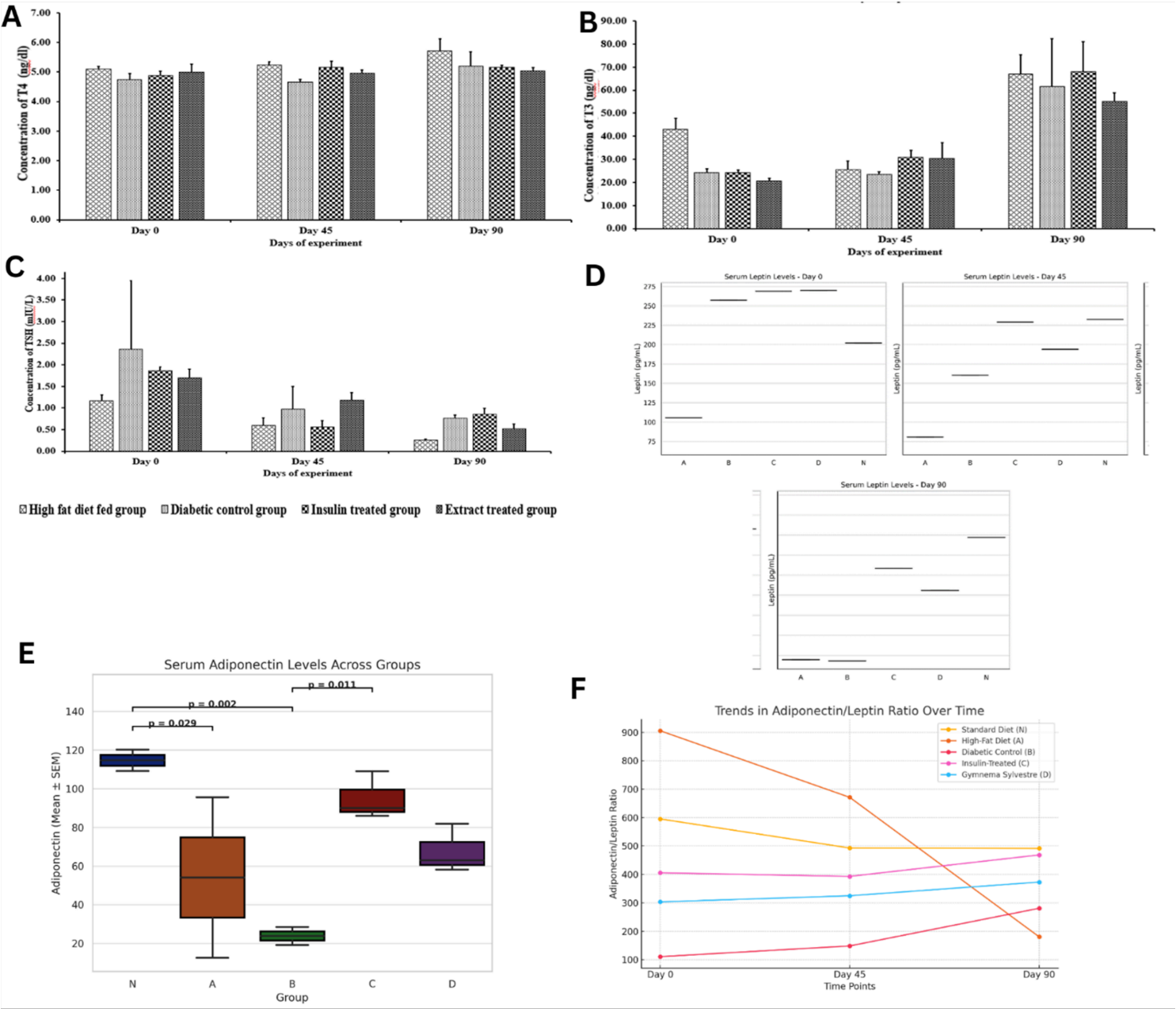


**Figure 3**

Treatment with *Gymnema sylvestre* leaf extract did not alter thyroid functions in diabetic rat model (A) Tetraiodothyronine or Thyroxine (T4). (B) Triiodothyronine (T3). (C) Thyroid Stimulating Hormone (TSH).

Data are presented as the mean ± SD (n=8)

(D) Effect of Insulin and *Gymnema sylvestre* leaf extract on serum leptin levels (pg/mL) in high-fat diet and Streptozotocin-induced Type 2 diabetic rats.

(E) Effect of Insulin and *Gymnema sylvestre* leaf extract on serum adiponectin levels (ng/mL) in high-fat diet and Streptozotocin-induced Type 2 diabetic rats.

(F) Effect of Insulin and *Gymnema sylvestre* leaf extract on A/L ratio in high-fat diet and Streptozotocin-induced Type 2 diabetic rats.

Data are presented as the mean ± SD (n=8). ###*p* < 0.001

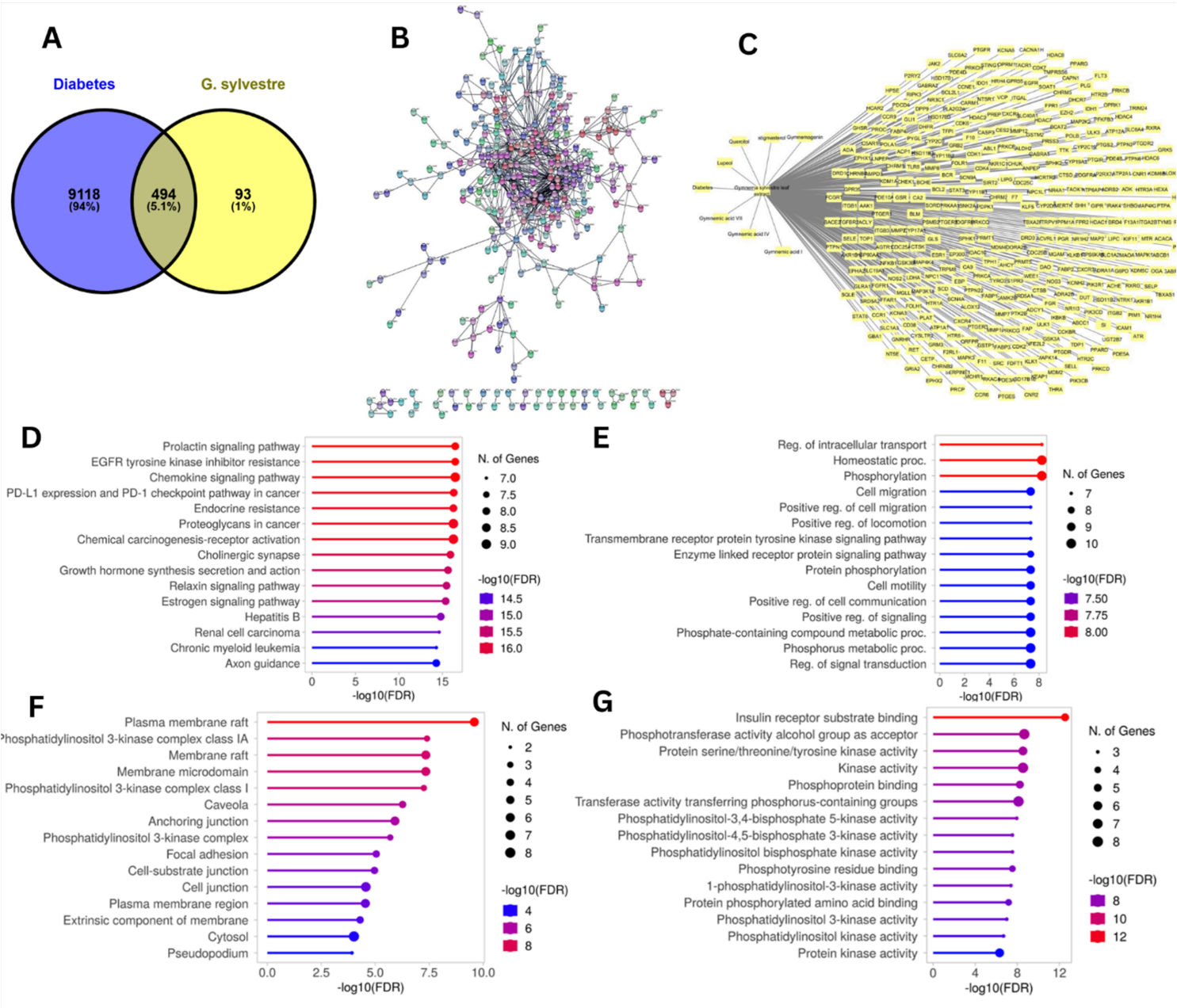


Figure 4

(A) Venn diagram showing *G. sylvestre* leaf extract and diabetes-related therapeutic targets. (B) Protein–protein interaction network of core targets constructed using STRING database. (C) Analysis of core targets using Cytoscape revealed 10 hub genes using the degree algorithm. (D-G) The KEGG enrichment analysis and potential mechanisms of the 10 candidate targets against diabetes.

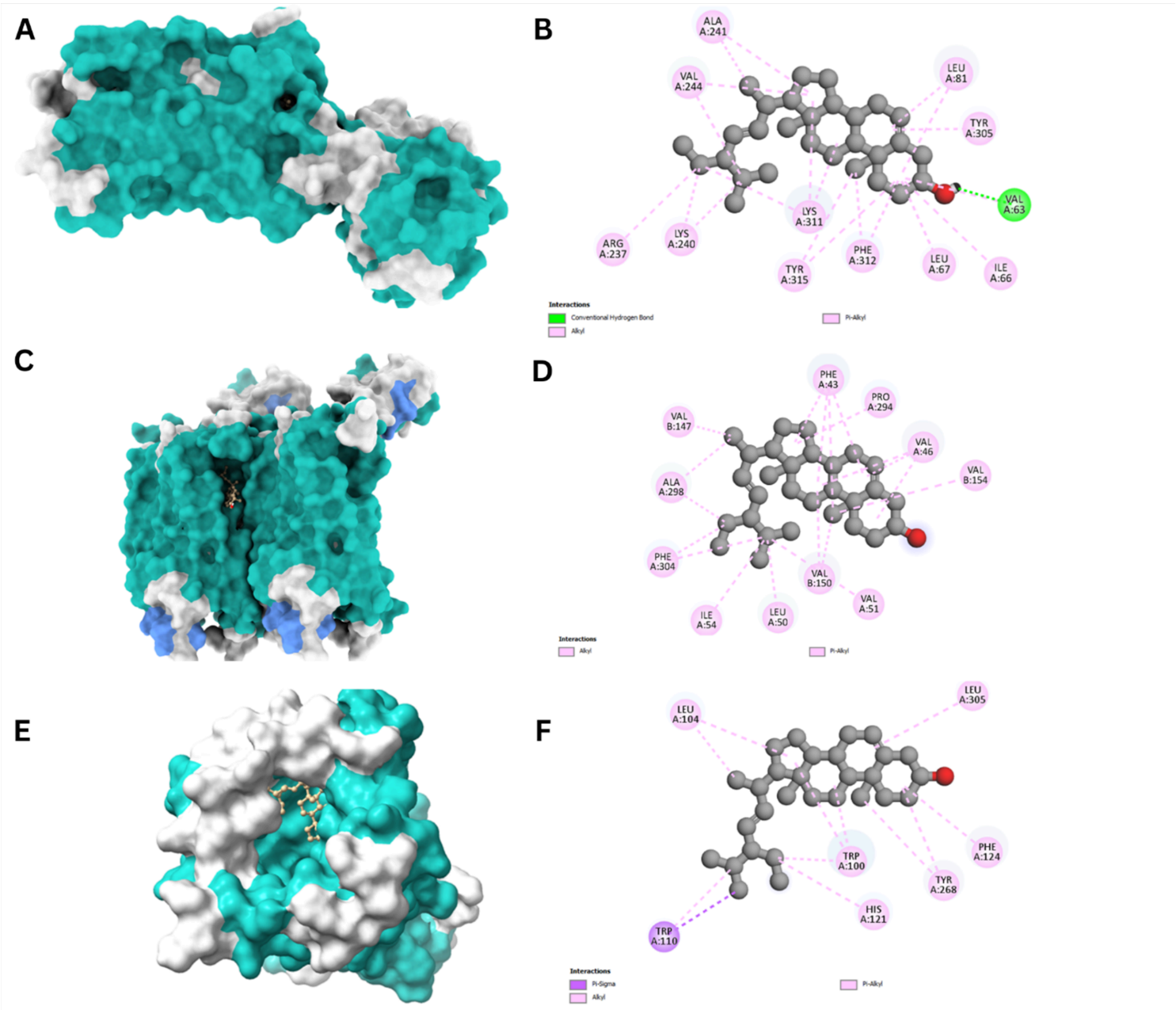


**Figure 5**

Illustration of protein and ligand complex, with the left side depicting three-dimensional interactions and right side depicting two-dimensional interactions between protein and ligand (A-B) Stigmasterol-CCR2. (C-D) Stigmasterol-CCR5. (E-F) Stigmasterol-CXCR7.

**Supplementary Files**

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