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Gymnemanol Modulates Lipid Metabolism and Adipogenic Gene Expression in 3T3-L1 Adipocytes

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Abstract

Background: Obesity is a metabolic disorder characterized by excessive adipose tissue accumulation and dysregulated lipid metabolism. Altered adipocyte differentiation and lipid metabolism contribute to obesity development. Medicinal plants-derived compounds have gained increasing attention as potential modulators of adipocyte function. Objective: This study aimed to evaluate the effects of gymnemanol (*Gymnema sylvestre*-derived compound) on adipocyte viability, triglyceride accumulation, and gene expression in lipid metabolism, adipogenesis, and adipokine regulation in 3T3-L1 adipocytes. Methods: 3T3-L1 preadipocytes were differentiated into mature adipocytes and treated with gymnemanol at non-cytotoxic concentrations determined by cell viability assay. Intracellular triglyceride content was quantified using a colorimetric assay. Gene expression analysis of lipogenic, adipogenic, lipolytic, and adipokine-related genes was performed. Results: Gymnemanol exhibited moderate cytotoxicity, and treatment significantly reduced intracellular triglyceride accumulation in a concentration-dependent manner. Gene expression analysis revealed lower lipogenic markers and differential modulation of adipogenic genes, with marked upregulation of Scd1 and altered expression of lipid droplet-associated genes. Lipolytic genes (Lipe, Lpl) were significantly increased, indicating enhanced lipid mobilization. Additionally, gymnemanol modulated adipokine expression, with relatively higher Adipoq levels compared to Lep and Ccl2, suggesting improved adipocyte metabolic signaling. Conclusion: Gymnemanol modulates adipocyte lipid metabolism by reducing triglyceride accumulation and altering the expression of genes involved in lipogenesis, lipolysis, adipogenesis, and adipokine signaling. These findings indicate a multi-target regulatory effect on adipocyte function. However, the study is limited to an *in vitro* model and mRNA-level analysis. Future work should include *in vivo* validation, protein-level confirmation, and mechanistic pathway studies to better define its therapeutic potential in obesity-related metabolic disorders.

Keywords

Gymnemanol, Obesity, Adipocyte Differentiation, Lipid Metabolism, Adipokine Signaling

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1. Introduction

Obesity is a chronic metabolic disorder characterized by excessive adipose tissue accumulation due to prolonged energy intake and energy expenditure imbalance (1-3). It is one of the serious public health concerns worldwide due to rapidly increasing prevalence and strong association with numerous metabolic complications. More than one billion people globally are living with obesity, and prevalence continues to rise across all age groups and socioeconomic settings (4,5). Obesity is recognized as a major risk factor in the development of

type 2 diabetes mellitus (6), cardiovascular diseases (7), hypertension (8), dyslipidemia (9), non-alcoholic fatty liver disease (10), and certain cancer types (11). Beyond its impact on individual health, obesity imposes a substantial economic burden due to increased healthcare expenditures, reduced productivity, and diminished quality of life (12). The pathophysiology of obesity is complex and involves interactions among genetic, environmental, behavioral, and metabolic factors (13-15). Despite advances in pharmacological and lifestyle-based interventions, long-term obesity management is challenging due to limited efficacy, adverse effects, poor treatment adherence, and weight regain following therapy (16-18). Consequently, there is growing interest in identifying novel therapeutic approaches that target the underlying mechanisms responsible for excessive lipid accumulation and metabolic dysfunction (19-21). A deeper understanding of the biological processes involved in obesity is essential to develop safe and effective strategies in managing this increasingly prevalent metabolic disorder.

Adipose tissue plays a vital role in the development of obesity. It was once considered a passive storage site for excess energy, but it is now recognized to regulate whole-body energy homeostasis (22-24). Adipose tissue stores extra energy in the form of triglycerides during nutrient abundance and releases fatty acids on energy demand (25-27). Adipose tissue expansion occurs *via* hypertrophy and hyperplasia, resulting from preadipocyte differentiation into mature adipocytes (28-31). Although these processes are essential to maintain energy balance, excessive adipocyte differentiation and lipid accumulation contribute to adipose tissue dysfunction and metabolic abnormalities (32). Adipogenesis is a tightly regulated process that involves transcriptional events that convert fibroblast-like preadipocytes into mature adipocytes (33-35). Concurrently, lipogenesis promotes the synthesis and storage of fatty acids and triglycerides, whereas lipolysis facilitates the hydrolysis of stored triglycerides to meet cellular energy requirements (36-39). Dysregulation of these processes leads to excessive lipid deposition, altered adipokine secretion, chronic low-grade inflammation, and insulin resistance (40-42). Thus, modulation of adipocyte differentiation and lipid metabolism is an attractive therapeutic strategy for obesity management. Compounds that limit triglyceride accumulation, suppress excessive lipogenesis, or enhance lipid mobilization restore metabolic balance and reduce obesity-related complications (43-48).

Adipocyte differentiation and lipid metabolism are regulated by transcription factors, metabolic enzymes, and adipokines. Among these, peroxisome proliferator-activated receptor gamma (PPARγ) is a master regulator of adipogenesis, governing the expression of numerous genes in adipocyte maturation and lipid storage (49,50). Several downstream targets contribute to the acquisition of mature adipocyte phenotype. Perilipin 1 (Plin1) regulates formation and stabilization of lipid droplets (51), whereas cell death-inducing DNA fragmentation factor α-like effector A (Cidea) participates in lipid droplet expansion and energy metabolism (52). Stearoyl-CoA desaturase 1 (Scd1) is a key enzyme in fatty acid desaturation that catalyzes conversion of saturated fatty acids into monounsaturated fatty acids that serve as important substrates for triglyceride synthesis and storage (53). Likewise, fatty acid synthase (FASN) plays a vital role in *de novo* lipogenesis by catalyzing synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA (54). In contrast to lipogenic pathways, lipid mobilization is regulated by lipolytic enzymes such as hormone-sensitive lipase (Lipe) and lipoprotein lipase (Lpl), which facilitate triglyceride hydrolysis and fatty acid utilization (55,56). Beyond their role in energy storage, adipocytes secrete adipokines that influence systemic metabolism. Adiponectin (Adipoq) enhances insulin sensitivity (57), whereas leptin (Lep) regulates appetite and energy expenditure (58). Chemokine ligand 2 (Ccl2) is associated with inflammatory responses and adipose tissue remodeling (59-61). Alterations in the expression of these genes can influence adipocyte function, lipid turnover, and metabolic homeostasis. Consequently, compounds modulating adipose tissue functions may offer promising therapeutic opportunities to manage obesity and associated metabolic disorders (62).

Growing interest in the development of safer and more effective anti-obesity therapies stimulated extensive research on bioactive compounds from medicinal plants. Natural products represent an important source of pharmacologically active molecules and have historically contributed to the discovery of numerous therapeutic agents (63-65). Several plant-derived compounds have been reported to regulate adipocyte differentiation, suppress lipid accumulation, enhance fatty acid oxidation, and improve metabolic homeostasis (66-69). *Gymnema sylvestre* R. Br. ex Schult. has attracted considerable attention because of its long history of use in traditional medicine for the management of diabetes and other metabolic disorders (70,71). Previous experimental studies demonstrated that extracts/isolated constituents of *G. sylvestre* can affect glucose metabolism, insulin sensitivity, lipid homeostasis, and body weight regulation (71-75). These findings suggest that *G. sylvestre* may possess therapeutic potential against obesity and associated metabolic abnormalities. However, cellular and molecular mechanisms underlying the activity of several individual constituents remain

insufficiently characterized. Consequently, further investigation is required to clarify its potential role in modulating adipocyte function and to assess its relevance as a candidate molecule to manage obesity-related metabolic disorders.

Based on the limited understanding of gymnemanol (Figure 1) effects on adipocyte biology, the present study was designed to investigate its role in regulating lipid metabolism and adipogenic function in differentiated 3T3-L1 adipocytes. Initially, gymnemanol cytotoxicity was evaluated to determine biologically relevant and non-toxic concentrations for subsequent experiments. Later, gymnemanol effect on intracellular triglyceride accumulation was assessed during adipocyte differentiation to examine its effect on lipid storage. To elucidate underlying molecular mechanisms, gene expression in lipogenesis, adipogenesis, lipolysis, and adipokine regulation was analyzed. This study aimed to provide insight into the regulatory effects of gymnemanol on adipocyte lipid metabolism and functional gene networks. The findings may contribute to understanding the biological activities of *Gymnema*-derived compounds and support its potential as a natural modulator of adipocyte function.

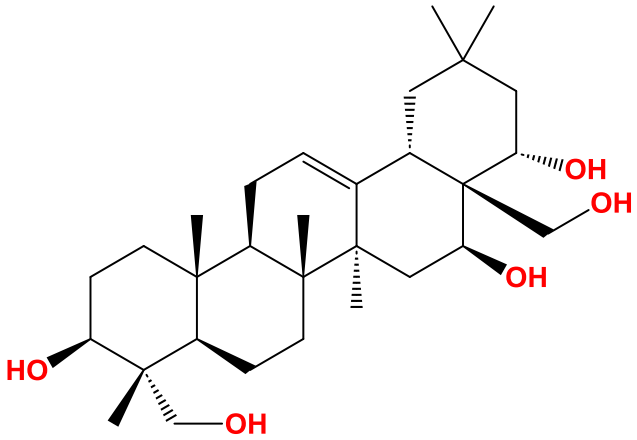

Figure 1: 2D structure of gymnemanol. Molecular formula: $C_{30}H_{50}O_5$, Molecular weight: 490.37, Number of hydrogen bond acceptors: 5, Number of hydrogen bond donors: 5, MolLogP: 3.20, MolLogS: -3.45 Log(moles/L), MolPSA: 81.19 A^2, MolVol: 610.30 A^3, pKa of most Basic/Acidic group: <0. / 15.00, blood-brain barrier permeability score: 1.73, Number of stereo centers: 11 (analyzed using Molsoft Druglikeness (https://molsoft.com/mprop/).

2. Materials and Methods

The detailed workflow of current study is outlined (Figure 2). Initially, 3T3L1 mouse embryonic fibroblast cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO_2 and subcultured before reaching 70% confluence (76-78). Adipocyte differentiation was performed as explained earlier (78). Post-confluent cells were cultured in DMEM containing 10% FBS, 1 µM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 10 µg/mL insulin. After 3 days, medium was replaced with DMEM supplemented with 10% FBS and 10 µg/mL insulin and refreshed every alternate day until mature adipocytes with visible lipid droplets were obtained. Differentiated adipocytes grown in 12-well plates were treated with gymnemanol. Cells were processed for RNA isolation and real-time PCR analysis. Next, the effect of gymnemanol on 3T3L1 cell viability was assessed using thiazolyl blue tetrazolium bromide (MTT) cytotoxicity assay (79,80). 3T3L1 cells were seeded in 96-well plates at a density of 5×10^3 cells/well and treated with gymnemanol for 24 h. MTT solution (10 µL; 5 mg/mL) was added to each well and incubated for 4 h. Formazan crystals were dissolved in 100 µL dimethyl sulfoxide, and absorbance was measured at 570 nm. Cell viability was calculated after blank correction and expressed as a percentage of control. Likewise, to determine triglyceride content, 3T3L1 cells cultured in 12-well plates were induced to differentiate and treated on day 1 of differentiation. Triglyceride content was quantified on days 4 and 8 using a colorimetric assay kit (Elabscience) according to the manufacturer's instructions. For gene expression studies, total RNA was extracted using TRIzol reagent (Sigma-Aldrich) according to the manufacturer's protocol with minor modifications wherever applicable. RNA pellets were dissolved in nuclease-free water, quantified using a NanoDrop spectrophotometer, and assessed for integrity by electrophoresis on a 1% agarose/formaldehyde gel containing 0.5 µg/mL ethidium bromide. First-strand cDNA was synthesized from 1 µg of total RNA using PrimeScript RT Reagent Kit. Quantitative real-time PCR was performed using SYBR Green PCR Master Mix. Primers are listed in Table S1. Amplification was carried out, and relative gene expression was calculated and normalized to β-actin. Amplification specificity was confirmed by melting curve analysis consisting of one cycle at 95°C for 1 min, 55 °C for 30 s, and 95 °C for 30 s (78,81). Statistical analysis was conducted using linear regression, one-way and two-way ANOVA, followed by appropriate post hoc tests wherever applicable.

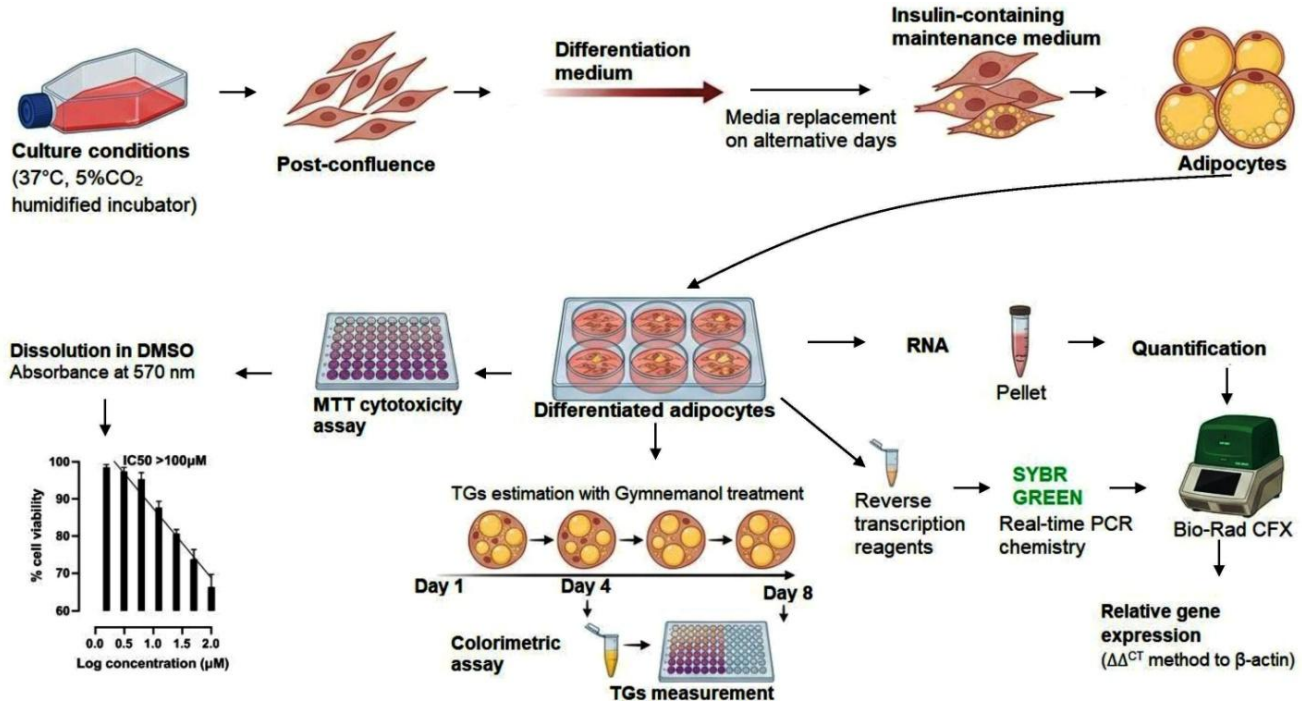

Figure 2: Pipeline to evaluate gymnemanol cytotoxicity, lipogenesis, and related gene expression. Experimental workflow for adipocyte cell culture, MTT cytotoxicity, triglyceride (TG) quantification, and relative gene expression.

3. Results

3.1. Cytotoxic effect of gymnemanol on 3T3-L1 cells

The cytotoxic effect of gymnemanol on 3T3-L1 cells was evaluated in the range of 1–100 µM. Gymnemanol induced a concentration-dependent reduction in cell viability, which is evidenced by a progressive decline in viable cells with increasing concentrations. At the lowest concentrations, cell viability remained close to 100%, whereas treatment with 100 µM reduced viability to 66%. Linear regression analysis demonstrated a significant negative relationship between gymnemanol concentration and cell viability, yielding the equation $y = -18.74x + 106.2$, where y represents percentage cell viability and x represents log concentration (µM). The slope of the regression line was significantly different from zero (slope = −18.74 ± 1.13; F = 277.0; df = 1,19; $p < 0.0001$). This indicates a statistically significant concentration-dependent cytotoxic effect (Table S2). The regression model showed a strong goodness of fit with an R^2 value of 0.9358 and a standard error of the estimate of 3.108. This demonstrates that approximately 93.6% of the variation in cell viability could be explained by changes in gymnemanol concentration. The estimated x-intercept (concentration) was 5.668 (95% confidence intervals (CI): 5.152–6.330), whereas the slope and y-intercept (% cell viability) CI were −21.10 to −16.38 and 103.3 to 109.2, respectively. Despite reduction in viability, cell survival remained above 50% across all tested concentrations (Figure 3). Consequently, inhibitory concentration (IC_{50}) was not achieved in the experimental concentration and was thus estimated to be greater than 100 µM. This indicates that gymnemanol exerts moderate cytotoxic effects on 3T3-L1 cells up to 100 µM and exhibits relatively low toxicity within the tested range.

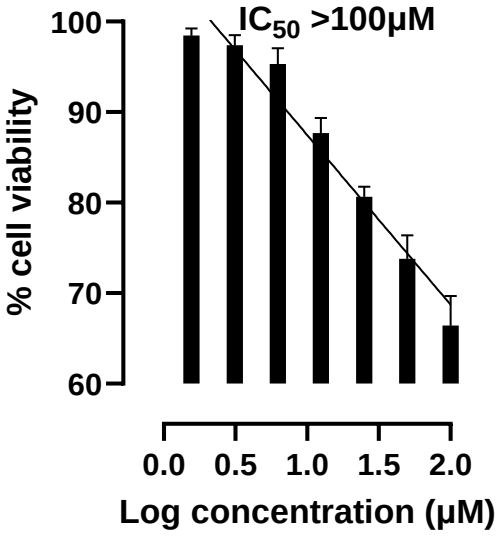

Figure 3: Gymnemanol cytotoxic effects. The IC_{50} was >100 µM. x-intercept = 5.668, 1/slope = -0.05336, F (DFn, DFd) = 277 (1,19), $y = -18.74x + 106.2$

3.2. Gymnemanol reduces intracellular triglyceride accumulation in adipocytes

Effect of non-toxic concentrations of gymnemanol (1.560, 3.125, and 6.250 μM) on intracellular triglyceride accumulation in adipocytes was evaluated on the 4th and 8th days of treatment (Figure 4). Two-way repeated-measures ANOVA (Table S3) revealed significant effects of treatment concentration, duration, and their interaction on triglyceride content. Interaction between concentration and time was statistically significant ($F(2,6) = 8.939$, $p = 0.0159$), indicating that the effect of gymnemanol on triglyceride accumulation depended on exposure duration. Importantly, significant effects were observed for concentration ($F(2,6) = 34.25$, $p = 0.0005$) and time ($F(1,6) = 49,799$, $p < 0.0001$). On day 4, triglyceride levels decreased progressively with increasing gymnemanol concentration. Post hoc Tukey's multiple comparison analysis (Table S4) showed significant reductions between all concentration groups. Triglyceride levels at 3.125 μM were significantly lower than those at 1.560 μM ($p = 0.0024$), whereas treatment with 6.250 μM produced further reduction compared with both 1.560 μM ($p < 0.0001$) and 3.125 μM ($p = 0.0010$). On day 8, triglyceride accumulation remained concentration-dependent, although the magnitude of differences among concentrations was smaller. Mean triglyceride levels were 356.8 mmol/L, 349.0 mmol/L, and 342.4 mmol/L for 1.560, 3.125, and 6.250 μM gymnemanol, respectively. Tukey's post hoc analysis revealed a significant reduction in triglyceride content at 6.250 μM compared to 1.560 μM ($p = 0.0010$). In contrast, differences between 1.560 and 3.125 μM ($p = 0.0508$) and between 3.125 and 6.250 μM ($p = 0.1018$) were not statistically significant. Comparison of treatment durations demonstrated a marked increase in triglyceride content from day 4 to day 8 across all concentration groups. Largely, mean triglyceride levels increased from 72.9 mmol/L on day 4 to 349.4 mmol/L on day 8, representing an average increase of 276.5 mmol/L (95% CI: 273.5–279.5 mmol/L). Despite this temporal increase associated with adipocyte maturation, gymnemanol decreased triglyceride accumulation in a concentration-dependent manner, with the strongest inhibitory effect at 6.250 μM. These findings indicate that gymnemanol significantly attenuates intracellular triglyceride accumulation in adipocytes, suggesting a potential anti-adipogenic or lipid-lowering effect. The effect was most pronounced at the highest tested concentration (6.250 μM), which produced the lowest triglyceride levels at both observation time points.

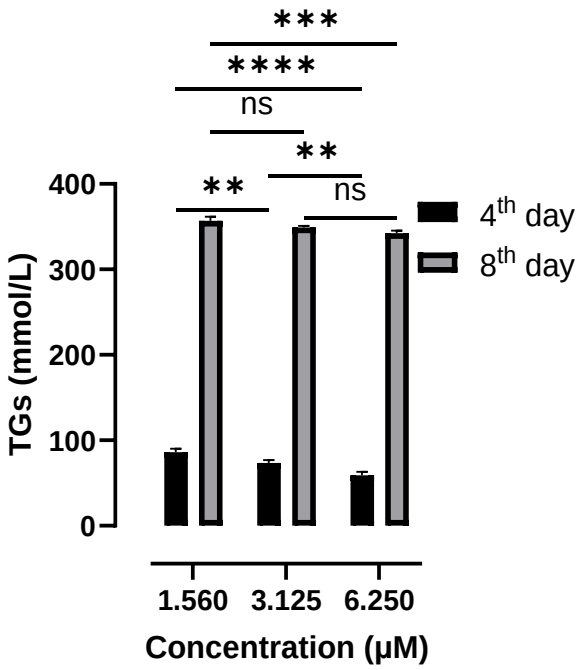

Figure 4: Effect of gymnemanol on triglyceride content of adipocytes. Concentrations were selected as they were non-toxic in the cytotoxicity assay. Data are represented as mean ± SD (n = 3). Data were analyzed by two-way ANOVA followed by multiple-comparison test. $^{ns}p>0.05$, $^{**}p<0.01$, $^{****}p<0.0001$. Detailed statistics are provided in Table S3 and S4.

3.3. Gymnemanol modulates the expression of genes involved in lipid metabolism

To elucidate the molecular mechanisms underlying the triglyceride-lowering potential of gymnemanol in adipocytes, relative mRNA expression levels of genes associated with lipid synthesis and lipid catabolism were analyzed. Expression of FASN and lipolytic markers, hormone-sensitive Lipe and Lpl, was quantified and normalized to β-actin (Figure 5). Analysis of gene expression data by one-way ANOVA (Table S5) demonstrated a statistically significant difference among 3 target genes ($F(2,6) = 23.45$, $p = 0.0015$), indicating distinct transcriptional responses in pathways regulating lipid metabolism. The calculated coefficient of determination ($R^2 = 0.8866$) showed that approximately 88.66% of the total variation in gene expression was attributable to differences among analyzed genes. Furthermore, the assumption of equal variances was met, as confirmed by Brown–Forsythe test ($F(2,6) = 0.4468$, $p = 0.6593$), supporting the validity of ANOVA results. Among evaluated genes, FASN exhibited the lowest expression level, with a mean normalized expression value of 1.367. In contrast, Lipe expression was

markedly elevated, reaching a mean value of 2.367, representing an approximately 1.73-fold higher expression level relative to FASN. Tukey's post hoc multiple comparison analysis confirmed that this increase was statistically significant (mean difference = 1.000, 95% CI: 0.074–1.926, $p = 0.0371$). The higher expression of Lipe suggests enhanced transcriptional activity of pathways involved in intracellular triglyceride hydrolysis and mobilization of stored lipids. The highest expression level was observed for Lpl, which displayed a mean normalized expression value of 3.433. Compared with FASN, Lpl expression was approximately 2.51-fold greater and differed significantly (Table S6) according to Tukey's multiple comparison test (mean difference = 2.067, 95% CI: 1.141–2.993, $p = 0.0012$). The magnitude of this difference represented the strongest statistical separation among all pairwise comparisons, indicating a pronounced predominance of Lpl transcript abundance relative to lipogenic marker FASN. Direct comparison between two lipolytic genes further revealed that Lpl expression significantly exceeded that of Lipe (mean difference = 1.067, 95% CI: 0.141–1.993, $p = 0.0285$). Quantitatively, Lpl expression was approximately 1.45-fold higher than Lipe, demonstrating a progressive increase in transcript abundance from FASN to Lipe and finally to Lpl. This graded expression pattern was clearly reflected in the gene expression profile, where the lowest transcript level was observed for lipogenic marker and the highest level for lipolytic marker. The expression pattern was characterized by lower abundance of FASN transcripts and substantially greater expression of Lipe and Lpl transcripts. The observed hierarchy of gene expression (FASN < Lipe < Lpl) indicates a transcriptional profile associated with enhanced lipid turnover and triglyceride metabolism in gymnemanol-treated adipocytes. Importantly, increased expression of lipolytic genes may correspond with the reduced intracellular triglyceride accumulation as observed in biochemical analysis. Moreover, modulation of genes involved in lipid catabolism may contribute to the lipid-lowering effects of gymnemanol in adipocytes.

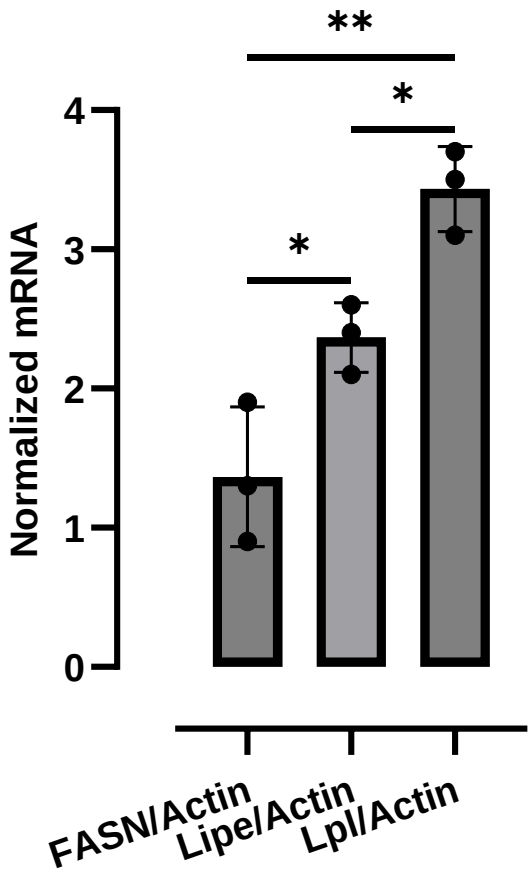

Figure 5: Effect of gymnemanol treatment on lipase enzyme coding genes expressed in 3T3L1 adipocytes. Treatment concentration (6.25 µM) was selected because it showed better lipogenesis. Data are represented as mean ± SD (n = 3). Data were analyzed by One-way ANOVA followed by a multiple-comparison test. *p<0.05, **p<0.01. Detailed statistics are provided in Table S5 and S6.

3.4. Gymnemanol alters lipogenesis-related mRNA expression

One-way ANOVA evaluated the effect of gymnemanol (6.25 µM) on the expression of genes associated with adipogenesis/lipogenesis in differentiated 3T3-L1 adipocytes. This concentration was selected based on its better lipogenic activity observed in preliminary experiments. The analysis included four adipogenic marker genes, PPARγ, Plin1, Scd1, and Cidea. ANOVA revealed a highly significant treatment effect among the analyzed genes ($F(3,8) = 113.2$, $p < 0.0001$), indicating substantial variation in gene expression levels. The model explained approximately 97.7% of total variability in the dataset ($R^2 = 0.9770$), demonstrating a strong treatment-

associated effect on adipogenic gene expression. Assessment of variance homogeneity using the Brown–Forsythe test showed no significant differences among group variances ($F(3,8) = 1.123$, $p = 0.3956$), confirming that the assumption of equal variances required for one-way ANOVA was satisfied. Gymnemanol treatment altered the expression pattern of adipogenic genes (Figure 6). Scd1 exhibited the highest expression, reaching approximately 45-fold normalized mRNA expression, which was significantly greater than the expression of other analyzed genes ($p < 0.0001$). Pairwise multiple comparison analysis demonstrated that Scd1 expression was significantly elevated compared with both PPARγ and Plin1 ($p < 0.0001$), indicating a pronounced stimulatory effect of gymnemanol on this lipogenic enzyme. Scd1 expression was significantly higher than Cidea ($p < 0.05$), although the magnitude of this difference was smaller than that observed with PPARγ and Plin1 (Table S7, S8). The expression of PPARγ and Plin1 remained relatively low and comparable, with normalized mRNA levels ranging between approximately 6–10 fold. Statistical analysis indicated no significant difference between these two genes ($p > 0.05$), suggesting that gymnemanol induced a similar transcriptional response for both markers. In contrast, Cidea expression was moderately increased (approximately 15–18 fold) and was higher than both PPARγ and Plin1 ($p < 0.0001$). However, Cidea expression remained significantly lower than Scd1 ($p < 0.05$), indicating that induction of Scd1 was more pronounced than other adipogenic genes. These findings demonstrate that gymnemanol (6.25 µM) differentially regulates adipogenic gene expression in 3T3-L1 adipocytes. The strongest transcriptional activation was observed for Scd1. The marked upregulation of Scd1 with moderate induction of Cidea and relatively modest expression of PPARγ and Plin1 suggests that gymnemanol may enhance pathways associated with fatty acid desaturation and lipid accumulation rather than uniformly stimulating all adipogenic markers.

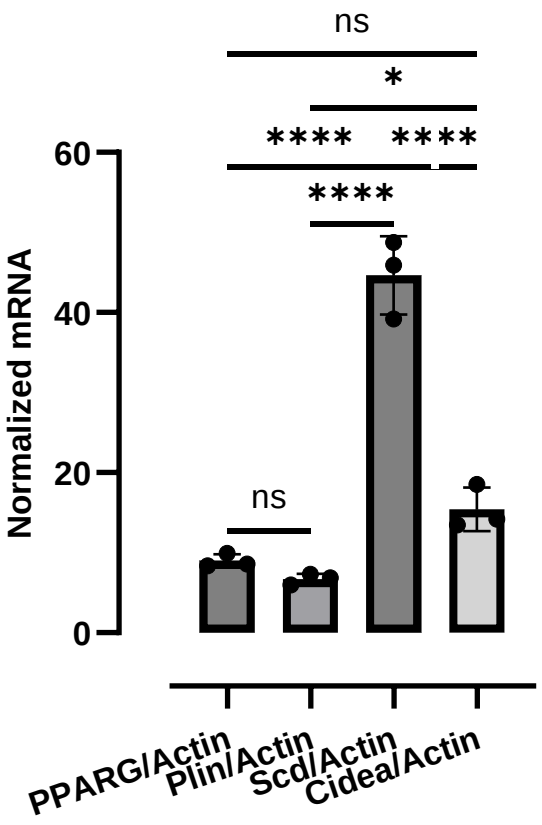

Figure 6: Effect of gymnemanol on adipogenesis-regulating genes in 3T3L1 adipocytes. Treatment concentration (6.25 µM) was selected because it showed better lipogenesis. Data are represented as mean ± SD (n = 3). Data were analyzed by One-way ANOVA followed by multiple-comparison test. $^{ns}p>0.05$, $*p<0.05$, $****p<0.0001$. Detailed statistics are provided in Table S7 and S8.

3.5. Effect of gymnemanol on adipokine gene expression in 3T3-L1 adipocytes

To determine whether gymnemanol alters adipocyte endocrine function, expression of representative adipokines (Adipoq, Lep, and Ccl2) was examined in differentiated 3T3-L1 adipocytes treated with gymnemanol (6.25 µM). One-way ANOVA (Table S9) revealed a significant difference in adipokine expression among analyzed genes ($F(2,6) = 7.729$, $p = 0.0219$), with the model explaining 72.0% of total variation ($R^2 = 0.7204$). Assumption of homogeneity of variance was satisfied, which was confirmed by the Brown–Forsythe test ($F(2,6) = 0.1121$, $p = 0.8957$). Adipoq exhibited the highest expression among multiple adipokines analyzed (Figure 7). Post hoc Tukey's multiple comparison analysis (Table S10) demonstrated Adipoq expression was significantly greater than Lep (mean difference =

2.933; 95% CI: 0.5838–5.283; p = 0.0202). In contrast, Lep showed the lowest expression level (3.86-fold), indicating a comparatively weaker transcriptional response. Although Adipoq expression was approximately 1.8-fold higher than Ccl2 expression (4.74-fold), this difference did not achieve statistical significance (p = 0.0811). Similarly, no significant difference was observed between Lep and Ccl2 expression (p = 0.5220). Predominance of Adipoq expression following gymnemanol treatment suggests enhanced adipocyte differentiation and maturation, as adiponectin is a hallmark marker of functional adipocytes. Relatively lower expression of Lep and intermediate Ccl2 expression indicate that gymnemanol does not uniformly stimulate all adipokine-related genes but appears to promote adiponectin-associated adipocyte functions.

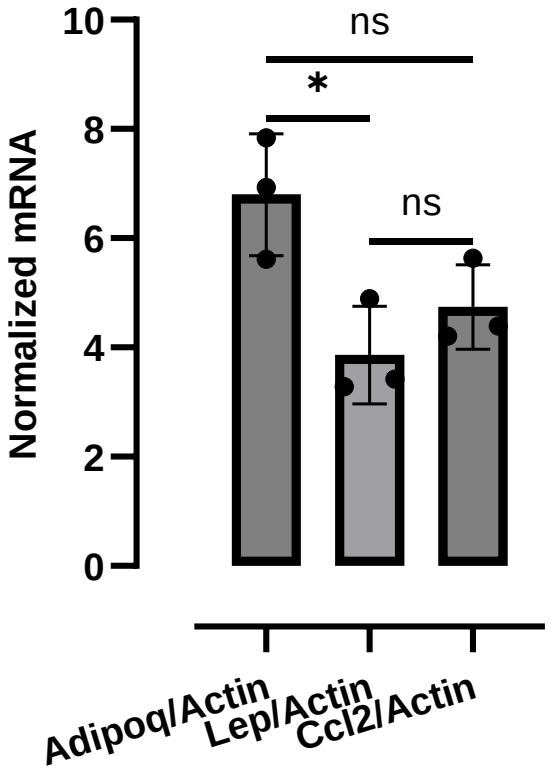

Figure 7: Effect of gymnemanol treatment on adipokine expression secreted by adipocytes. Treatment concentration (6.25 µM) was selected because it showed better lipogenesis. Data are represented as mean ± SD (n = 3). Data were analyzed by One-way ANOVA followed by multiple-comparison test. $^{ns}p>0.05$, $*p<0.05$. Detailed statistics are provided in Table S9 and S10.

4. Discussion

The present study demonstrates that gymnemanol exerts a modulatory effect on adipocyte lipid metabolism and gene regulation in differentiated 3T3-L1 cells. The findings indicate that gymnemanol influences multiple interconnected aspects of adipocyte function, intracellular lipid accumulation, and transcriptional regulation of lipid-regulating enzymes and adipokines (Figure 8). Gymnemanol appears to induce a broader metabolic reprogramming in adipocytes by altering the balance between lipid storage and lipid mobilization pathways. This integrated response suggests that gymnemanol may act at upstream regulatory levels controlling adipocyte metabolic phenotype, influencing both structural lipid deposition and functional endocrine output. A notable aspect of observed effects is the apparent shift in cellular lipid modulation, where reduced triglyceride accumulation coincides with changes in gene expression profiles linked to both lipogenic and lipolytic processes. This implies that gymnemanol does not simply inhibit lipid storage in a passive manner, but may modulate the dynamic equilibrium between lipid synthesis and breakdown. Such a response is consistent with the concept of adipocyte metabolic plasticity, where adipocytes adjust their functional state in response to external bioactive signals (35,82-84). In this context, gymnemanol may contribute to a transition toward a less lipid-loaded and more metabolically responsive adipocyte phenotype. In addition, observed alterations in adipokine gene expression suggest that gymnemanol not only affects intracellular metabolic pathways but also endocrine characteristics of adipocytes. Since adipokines serve as key mediators of systemic metabolic regulation (85-87), changes in their expression indicate that the compound may have implications extending beyond cellular lipid storage, affecting broader metabolic signaling.

Findings of the present study are consistent with previous reports describing the metabolic effects of plant-derived bioactive compounds on adipocyte differentiation and lipid accumulation (67,69,88-90). Literature indicates that phytochemicals can interfere with adipogenesis and lipogenesis by targeting key regulatory pathways in 3T3-L1 adipocytes, resulting in reduced intracellular triglyceride storage and altered expression of genes involved in lipid metabolism (91-93). In this context, observed effects of gymnemanol align with prior studies reporting metabolic benefits of *G. sylvestre* extracts/constituents (72,73,75,94). Earlier investigation demonstrated *Gymnema* to improve glucose tolerance (95), influence lipid homeostasis (96), and reduce obesity-related markers (97) in experimental systems. Although many studies focused on crude extracts or well-

characterized gymnemic acids, the present findings extend this evidence by implicating gymnemanol as an active contributor to metabolic effects attributed to *G. sylvestre*. The pattern observed in this study is in agreement with established concepts in adipocyte biology, where suppression of lipid accumulation is accompanied by changes in gene networks regulating fatty acid synthesis, lipid droplet formation, and lipid mobilization (98-101). The concurrent modulation of these pathways has been reported for other natural compounds with anti-obesity potential, reinforcing the idea that effective metabolic regulation typically involves integrated effects on multiple nodes in adipocyte regulatory circuits (47,102,103). Accordingly, present results support broader literature suggesting that plant-derived compounds can exert meaningful metabolic effects through coordinated regulation of adipocyte function rather than single biochemical inhibition.

Although present findings are consistent with the general anti-adipogenic effects reported for several plant-derived compounds (90), certain aspects of the observed response pattern suggest a context-dependent mode of action for gymnemanol. Phytochemicals primarily suppress both lipid accumulation and adipogenic gene expression (92). The current results indicate a more differentiated regulatory profile across lipid metabolism pathways. Transcriptional responses do not reflect a simple global downregulation of adipocyte differentiation markers, but rather a selective modulation of genes associated with lipid-modulating and adipocyte functional state. This pattern may represent a functional distinction between gymnemanol and other well-characterized anti-adipogenic agents. Instead of only inhibiting adipocyte differentiation programs, gymnemanol appears to differentially influence components of lipid metabolism, suggesting partial reprogramming over complete suppression of adipogenic signaling. Such a profile may reflect interaction with upstream regulatory nodes that exert differential control over downstream metabolic branches, leading to pathway-specific rather than global transcriptional effects. Furthermore, observed dissociation between lipid accumulation and certain adipogenic marker expression patterns extends current understanding of how *Gymnema*-derived constituents may modulate adipocyte biology. Previous reports have largely focused on bulk extracts or glycosidic compounds, which produce more generalized inhibitory effects on adipogenesis (74,75,104,105). In contrast, present data suggest that gymnemanol may exert a more selective influence, targeting enzymatic or transcriptional processes in lipid metabolism. This divergence is notable as it suggests that gymnemanol may not solely function as an anti-adipogenic suppressor but also function as an adipocyte metabolic state modulator. Such a mechanism could implicate how metabolic flexibility is regulated at the cellular level, and help to explain differences between whole-extract effects and isolated compound activity.

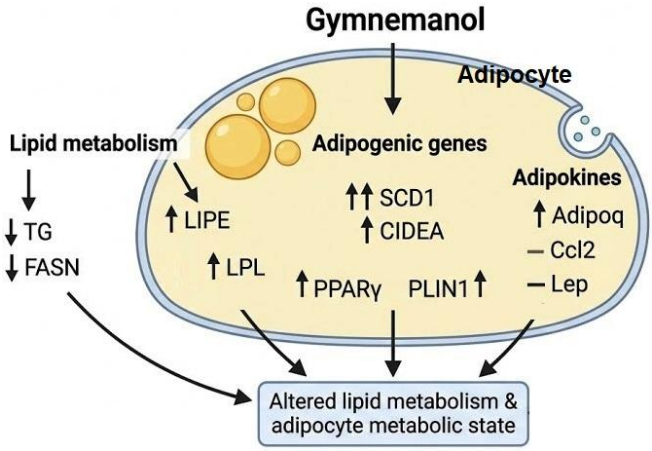

Figure 8: Gymnemanol-mediated modulation of lipid metabolism and adipocyte gene expression in 3T3-L1 adipocytes. Gymnemanol may regulate metabolic reprogramming/metabolic plasticity, with reduced triglyceride accumulation, enhanced expression of lipid-mobilization genes, differential adipogenic gene expression, and altered adipokine expression.

The effects of gymnemanol on adipocyte lipid metabolism and gene expression patterns can be interpreted in the paradigm of adipocyte transcriptional regulation and metabolic plasticity. Adipocyte differentiation and lipid handling are governed by hierarchical transcriptional networks (106,107). Changes observed in both lipid accumulation and gene expression suggest that gymnemanol may act at or upstream of these regulatory control points, thereby affecting multiple downstream metabolic processes simultaneously. One plausible interpretation is that gymnemanol modulates signaling pathways that converge on key transcriptional regulators of adipogenesis and lipid metabolism. Such upstream modulation would be expected to produce broad but non-uniform changes in gene expression, which is consistent with the differential responses observed among lipogenic, lipolytic, and adipokine-related genes. In adipocytes, these regulatory systems are interconnected, and relatively modest shifts in signaling can result in measurable changes in metabolic output, including altered triglyceride storage and lipid turnover dynamics (108-110). From a metabolic standpoint, reduction in intracellular lipid accumulation alongside changes in lipid-handling gene expression may reflect a shift in the balance between anabolic and catabolic processes within adipocytes (111-113). This suggests a reprogramming of metabolic flux rather than complete shutdown of adipogenic activity. Such shift is consistent

with partial modulation of adipocyte functional state in which cells may retain viability and differentiation, but exhibit altered metabolic behavior. This interpretation aligns with the broader concept that adipocytes exist along a continuum of functional states rather than discrete on/off differentiation endpoints (114,115). Additionally, modulation of adipokine-associated gene expression suggests that gymnemanol may not only modulate intracellular metabolic pathways but also extracellular signaling functions of adipocytes. Due to the role of adipokines in systemic metabolic regulation, this may indicate that the compound affects metabolic signaling that extends beyond lipid storage. These mechanistic considerations support the view that gymnemanol acts as a multi-level regulator of adipocyte biology, regulating both intracellular metabolic pathways and broader transcriptional programs governing adipocyte function.

The effects of gymnemanol on adipokine gene expression provide additional insight into how adipocyte metabolic changes may translate into broader endocrine and physiological consequences. Adipocytes are not only energy storage cells but also active units that secrete adipokines regulating systemic processes (116-118). Thus, alterations in adipokine expression reflect functional changes that extend beyond intracellular lipid metabolism and may manipulate whole-body metabolic regulation (119). In the present study, observed pattern of adipokine gene modulation suggests a shift toward a more metabolically favorable adipocyte signaling profile. The relative predominance of adiponectin-associated expression is relevant, as adiponectin is widely recognized for its insulin-sensitizing (120,121) and anti-inflammatory (122) properties. Increased adiponectin-related signaling is associated with improved metabolic efficiency and reduced risk of metabolic dysfunction (123-125), indicating that gymnemanol may support a more physiologically active adipocyte phenotype despite changes in lipid storage dynamics. At the same time, comparatively lower expression of leptin-related signaling suggests that gymnemanol does not uniformly activate all adipokine pathways, but exerts selective effects on adipocyte endocrine output. Changes observed in inflammatory-related adipokine expression suggest that gymnemanol may modulate adipose tissue inflammation. From a physiological perspective, such differential adipokine regulation is significant because it indicates potential downstream effects on systemic metabolic homeostasis. Therefore, effects observed here may represent an important mechanistic link between cellular metabolic actions of gymnemanol and its potential systemic metabolic implications. These findings support the view that gymnemanol modulates adipocyte endocrine function in a selective and functionally relevant manner.

A key focus of this study is its integrated experimental design, which combines functional assays with molecular analyses to characterize the effects of gymnemanol on adipocyte biology. By assessing cytotoxicity, intracellular triglyceride accumulation, and gene expression in the same cellular model, the study provides a multi-layered evaluation of both phenotypic and mechanistic outcomes. This approach reduces reliance on a single endpoint and allows more robust interpretation of how gymnemanol influences adipocyte function across different biological scales. The use of 3T3-L1 adipocyte differentiation (126) system further strengthens the experimental paradigm, as this model is well-established and accepted for studying adipogenesis and lipid metabolism. Its physiological relevance in adipocyte research enhances the comparability of findings with existing literature. Another strength is the inclusion of both lipogenic and lipolytic gene panels (127), along with adipokine-related markers (128), which allows more comprehensive assessment of adipocyte functional state. This broad gene selection provides insight into multiple metabolic pathways rather than focusing on a single regulatory axis. Integration of these molecular endpoints with biochemical triglyceride measurements strengthens mechanistic inference by linking transcriptional changes with functional metabolic outcomes. Finally, focusing on a defined phytochemical constituent rather than a crude extract improves specificity and interpretability (129). This reduces confounding from compound mixtures and allows clearer attribution of observed biological effects to gymnemanol itself.

Despite the strengths of study, we acknowledge some limitations of present study. First, the findings are based solely on an *in vitro* 3T3-L1 adipocyte model, which does not fully reflect the complexity of adipose tissue and systemic metabolic regulation *in vivo* (76,130). Second, study relied primarily on mRNA expression data without protein-level or functional validation, limiting conclusions about biological significance of observed gene expression changes. Third, molecular mechanisms underlying gymnemanol effects were not directly investigated, leaving its precise mode of action unclear. Fourth, concentrations tested may not represent physiologically achievable levels, and absence of pharmacokinetic and long-term exposure data limits translational interpretation. Although standardized protocols were used, minor experimental variability cannot be completely excluded. Future studies involving *in vivo* models, protein-level validation, and mechanistic analyses are needed to confirm the biological and therapeutic relevance of gymnemanol. Key priorities

include validating its effects via *in vivo* models of obesity and metabolic dysfunction to assess impacts on body weight, insulin sensitivity, and lipid metabolism. Further studies should investigate the signaling pathways underlying its effects on adipocyte function and confirm gene expression findings through protein-level and enzyme activity. Dose–response relationships, pharmacokinetics, long-term safety, and chronic exposure effects are also needed. Comparative studies with other *Gymnema*-derived compounds may determine if gymnemanol has unique biological activity.

5. Conclusion

This study investigated the effects of gymnemanol on adipocyte lipid metabolism and associated genes using an *in vitro* 3T3-L1 adipocyte model. Experiments combined cell viability assessment, quantification of intracellular triglyceride, and evaluation of key lipogenic, lipolytic, and adipokine-related gene expression. It reduces intracellular triglyceride accumulation and induces marked changes in gene expression in lipid metabolism and adipokines. These findings suggest that gymnemanol exerts a multi-target regulatory effect on adipocyte biology, promoting a shift toward altered lipid handling and a potentially more metabolically active cellular phenotype. However, study is limited by *in vitro* design, reliance on mRNA-level data without protein validation, and absence of *in vivo* confirmation. Additionally, pharmacokinetic relevance and long-term biological impact remain unaddressed. Future research should focus on validating these findings in animal models. This will elucidate signaling pathways and confirm gene expression changes at protein functional activity. It will help better define the therapeutic potential and molecular mechanism of gymnemanol in metabolic diseases.

Author contribution

DS; Data Curation, Draft, Review, Edit, Revise, BB; Review, Edit, Draft, Experimentation, Analysis

Declaration of interests

All authors of this manuscript declare that they do not have any conflicts of interest by any means.

Ethical statement

This study does not include any human participation or animal use.

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Availability of data and materials

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