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A Triterpene Glycoside Fraction, TG from *Gymnema sylvestre* Ameliorates Insulin Resistance by Stimulating Glucose Uptake in 3T3L1 Adipocytes and C2C12 Skeletal Muscle Cells

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Abstract

Type 2 diabetes mellitus (T2DM) which is characterized by insulin resistance in muscle and adipose tissues is a major problem worldwide. Plant based medications are well known from ancient times for possessing antidiabetic properties. Amongst them, *Gymnema sylvestre* (GS) is one such antidiabetic medicinal plant which has been used traditionally over the years for the treatment of T2DM. The aim of the present study was to investigate the effect of triterpene glycoside (TG), an active fraction isolated from ethanolic extract of *Gymnema sylvestre* (EEGS) on a battery of targets; glucose transporter (GLUT-4), peroxisome proliferator activator receptor gamma (PPAR-γ), adiponectin and leptin involved in glucose transport and metabolism. No cytotoxic effects were observed in treated cells up to 600 µg/ml of TG as measured by MTT and ROS assays. Elevation of GLUT-4 and PPAR-γ by TG in association with glucose transport supported the upregulation of glucose uptake at concentrations of 50 and 100 µg/ml respectively. Additionally, TG showed higher expression of adiponectin and leptin, confirming the favorable pharmacological effect of TG on insulin resistance. The results were comparable to the known antidiabetic drug pioglitazone and commercial standard DAG. Thus TG could be considered as a safe nutraceutical candidate/functional phytoingredient in amelioration of insulin resistance.

Keywords

Insulin Resistance, *Gymnema sylvestre*, GLUT-4, PPAR-γ, Glucose Uptake

1. Introduction

Type 2 Diabetes mellitus (T2DM) is a well-known metabolic disorder categorized by prolonged hyperglycemia, in association with the defective insulin secretion leading to insulin resistance in major glucose utilizing tissues like skeletal muscle and adipose tissues [1]. While conventional treatments such as sulfonylureas, metformin and thiazolidinedones are effective, they have several limitations; including adverse side effects, the inability to halt further loss of insulin secretory capacity [2]. In this view, plant-based medicines have better benefits over synthetic class of drugs. In current times, many researchers have found that various phytoconstituents are highly promising in the treatment of diabetes [3]. More than 1200 plant species are reported worldwide as antidiabetic [4] [5] [6]. Amongst them 400 have been scientifically evaluated for T2DM treatment [7]. Current studies have demonstrated that supplementation with natural antioxidant products improve hyperglycemic status by reducing the oxidative stress [8]. Medicinal herbs contain various bioactive compounds and thus can unveil manifold influence on insulin production as well as discrete mechanisms of insulin actions such as; insulin-sensitizing, insulin mimicking, inhibition of intestinal carbohydrate digestion and absorption. Insulin sensitizers include medicinal plant extracts that increase glucose disposal and uptake by muscle, hepatic and fat cells as well as those that control hepatic glycogen metabolism [7].

Gymnema sylvestre (GS) is one such traditional medicinal plant which is studied by researchers for its antidiabetic and hyperglycemic properties [9]. Nevertheless, the molecular mechanisms towards the antidiabetic activity exhibited by *Gymnema sylvestre* still remain uncertain. In our previous work, we have isolated and completely characterized triterpene glycoside fraction (TG) containing four potential active principles from *Gymnema sylvestre* [10]. In this particular study, we have reported the *in vitro* antidiabetic effects of TG on C2C12 skeletal muscle and 3T3L1 adipocyte cell line models.

During food consumption, glucose is channelled mainly in the skeletal muscle and to a lesser extent in fat tissues and liver. Insulin-stimulated glucose uptake takes place in muscle and adipocytes by signalling the translocation of GLUT-4 glucose transporters from intracellular membranes on to the cell surface [11]. In type 2 diabetes, insulin resistance is manifested by decreased glucose transport and impaired metabolism in adipocytes and skeletal muscle, resulting in down-regulation of the significant insulin-responsive GLUT, GLU-4 [12]. Additionally, PPAR-γ (a transcription factor fitting to the nuclear receptor superfamily) plays an important role in insulin-induced glucose uptake. The part of PPAR-γ in the development of insulin resistance has been scientifically well established. Therefore, the drugs that enhance PPAR-γ expression plays a vital role in reversing the insulin resistance [12] [13] [14] [15]. Further, leptin and adiponectin are the essential insulin-sensitizing adipokines secreted by adipocytes [16]. Adiponectin augments insulin sensitivity and benefits in controlling dyslipidemia through AMPK activation and amplified fatty acid (FA) oxidation [17] [18]. Briefly, the scientific

literature proposes that GLUT-4, PPAR-*γ*, adiponectin and leptin levels have a positive connection in governing the blood glucose levels and insulin resistance in patients suffering from type 2 diabetes and its complications. Considering the possible antidiabetic property of *Gymnema sylvestre* (GS), a plant extensively used in ayurvedic medicine against diabetes, an attempt was made to discover the possible correlation between the antidiabetic activity of isolated active fraction TG from *Gymnema sylvestre* and gene expressions of GLUT-4, PPAR-*γ*, adiponectin and leptin *in vitro*. This would provide us further insight into the molecular mechanisms exerted by TG towards the antidiabetic activity.

2. Materials and Methods

2.1. Chemicals and Reagents

The dried leaf powder of Gymmnema Sylvestre (GS) was obtained from Nikhila Karnataka Central Ayurvedic Pharmacy (Mysore, India). 3-4,5-(Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from SRL chemicals (Bangalore, India). Mouse fibroblast preadipocyte cells (3T3L1) and C2C12 skeletal muscle cell lines was bought from NCCS (Pune, India) cell repository. Dulbecco's minimal essential medium (DMEM), Fetal Bovine Serum (FBS), antimycotic solution were purchased from Himedia chemicals (Bangalore, India). CDNA kit was from Thermo Scientific (USA). The glucose uptake fluorimetric kit, acridine orange (AO), 2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA), insulin, pioglitazone, Deacylgymnemic acid (DAG), cell culture supplements, TRI reagent, and reagents used were of molecular biology grade and procured from Sigma-Aldrich (St. Louis, USA). All other chemicals used in our study were of analytical grade and purchased from Himedia chemicals (Bangalore, India).

2.2. Plant Material Preparation

The plant material was prepared as per our previously published work [10]. The fraction (III), termed as TG exhibiting maximum activity was chosen for the current *in vitro* antidiabetic studies. TG has been identified as a mixture of triterpene glycosides has been completely characterized.

2.3. Cell Culture

3T3L1 preadipocyte and C2C12 skeletal muscle cell lines were obtained from NCCS, Pune and maintained under 5% CO_2, 37˚C in DMEM media supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml of penicillin and 0.1 mg/ml streptomycin. For differentiation, C2C12 cells were transferred to DMEM with 2% FCS, 4 - 6 days after confluence. The extent of differentiation was established by observing multinucleation of cells and 90% fusion of myoblasts into myotubes were considered for our study.

Differentiated myotubes were incubated with test samples along with controls in media for 24 h, respectively and then analysis was performed. For 3T3L1 cells, after achievement of confluence, the growth medium was replaced with adipo-

genesis initiation media (0.5 mm 3-iso butyl-1-methyl xanthine, 10% FBS and 1 µl dexamethasone in DMEM). Primarily, the cells were incubated for 48 h, after which adipogenesis initiation media was replaced by adipogenesis progression media (DMEM with 10% FBS and 10 µm/ml insulin). Sequentially, the plate was incubated for 48 h, and then adipogenesis progression media was removed. The cells were treated with various concentrations of test extracts along with controls in adipogenesis maintenance media (DMEM with 10% FBS). The test samples were dissolved in DMEM media and incubated with adipogenesis maintenance media.

2.4. Cell Viability Assay

3-4,5-(Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was based on the protocol described [19]. C2C12 and 3T3L1 cells were seeded at a density of 2×10^4 cells/well in 96-well plates for 24 h. Then the cells were treated with various concentrations of active tritepene glycoside fraction (TG) and ethanolic extract of *Gymnema sylvestre* (EEGS) (20 - 600 µg) and incubated for 24 h. After the required period of treatment, the media was removed, MTT solution [10 µl; 5 mg/ml MTT in phosphate-buffered saline (PBS)] was added and incubation was continued for additional 3 h. After this, the media was removed and to the treated cells DMSO was added. Incubation was continued for another 30 min in the dark. The cell viability was then recorded at 570 nm.

2.5. Intracellular ROS

The quantification of the cellular antioxidant activity was estimated as per the the method described using 2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The cells were seeded in a 96-well plate at a density of 5×10^4 cells/well. After 24 h of incubation period, the cells were treated with 20, 35, 50 and 100 µg concentrations of TG and EEGS separately and incubated further for 24 h. The intracellular oxidation of DCFH-DA in the wells was estimated using fluorescence reader at excitation (488 nm) and emission (525 nm).

2.6. Fluorescence Microscopy of MIN6 Cells

C2C12 cells were seeded at a density of 1×10^5 cells/ml on poly-L-Lysine coated cover slips in six well plates comprising DMEM for 24 h and then differentiated as per the protocol mentioned previously. Cells were stained using the fluorescent dye acridine orange (AO). The images were then taken using a fluorescence microscope (Nikon, Tokyo, Japan) [20].

2.7. Glucose Uptake Assay

The fully differentiated C2C12 cells and 3T3L1 adipocytes (2×10^4 cells/well) were serum deprived for overnight and then washed with HEPES in KREB's Ringer phosphate solution (KRP buffer). The cells were later incubated for 30 min at 37˚C with KRP buffer supplemented with 0.1% bovine serum albumin.

This procedure was followed by sample treatment with EEGS and TG at 50 and 100 µg/ml and commercial standard Deacylgymnemic acid (DAG, 50 µg/ml) and antidiabetic drug Pioglitazone (100 µg/ml) separately along with vehicle controls in 96 well plates. 2 Deoxy-glucose (2-DG) solution was added to all the wells and incubated for 30 min at 37˚C. Subsequently, the liquid m-edium was aspirated from all the wells to stop the glucose uptake. The cells were then washed twice with cold KRP buffer solution. Further, the cells were lysed with 0.1 M NaOH solution, and an aliquot of the cell lysates were used to estimate the cell-associated glucose. Glucose uptake was measured by Glucose uptake fluorimetric kit (Sigma, USA). Three independent experimental values in replicates were taken to determine the percentage increase of glucose uptake over controls [21].

2.8. RT-Polymerase Chain Reaction (RT-PCR)

RT-PCR was carried out according to [22], with slight modifications. Post confluence, media was removed and cells were washed twice with ice cold PBS. In the next step, cells were lysed using TRIZOL reagent and proteins were extracted using chloroform. The total RNA was precipitated using isopropanol and the RNA precipitate was washed with 70% ethanol. The pellet was then resuspended in 50 µl of DEPC-treated water. Reverse transcription was carried out using cDNA synthesis kit (Thermo Scientific, USA Cat. No K1621). The primers used were as follows, GLUT-4: Sense, 5'-CGGGACGTGGAGCTGGCCGAGGAG-3'; anti-sense, 5'-CCCCCTCAGCGAGTGA-3' (318-bp), PPAR-*γ*; sense, 5'-GGATTCATGACAGGGAGTTCCTC-3'; anti-sense, 5'-GCGGTCTCCACTGAGAATAATGAC-3' (155-bp); adiponectin: Sense, 5'-GTTCTACTGCAACATTCCGG3'; antisense, 5'-TACACCTGGAGCCAGACTTG-3'; leptin: Sense, 5'-TGTGCTGCAGATAGCCAATG-3'; Antisense, 5'-AGGGAGCAGCTCTTGGAGAAG-3'glyceraldehydes-3-phosphate dehydrogenase (GAPDH); sense, 5'-CCACCC ATGGCAAAT. TCCATGGCA-3'; anti-sense, 5'-TCTAGACGGCAGGTCAGG TCCACC-3' (588-bp) [23]. For RT-PCR (20 µl) reaction, 10 µl of Hi-SYBR Master Mix (with Taq Polymerase), 1 µM of paired primers, Template DNA (5 µl of <250 ng) was made up to 20 µl with molecular biology grade sterile water. The RT-PCR conditions were as follows: 25 cycles of 94˚C for 3 min, 94˚C for 30 s, 60˚C for 30 s and 72˚C for 45 s. Triplicate reactions were set up for each sample, and the mRNA abundance was normalized to eukaryotic 18 s ribosomal RNA expression. No template controls (NTC) were used as negative controls for each gene. Assays were carried out in 96-well applied for biosystem plates and were covered with optical adhesive (P/N 4346906 and P/N4311971; ABI). The 2-ΔΔCT method was used to calculate the relative gene expression.

Statistical Analysis

All the values were expressed as mean ± SD. The results were analyzed statistically using two-way ANOVA followed by Tukey's multiple comparison tests

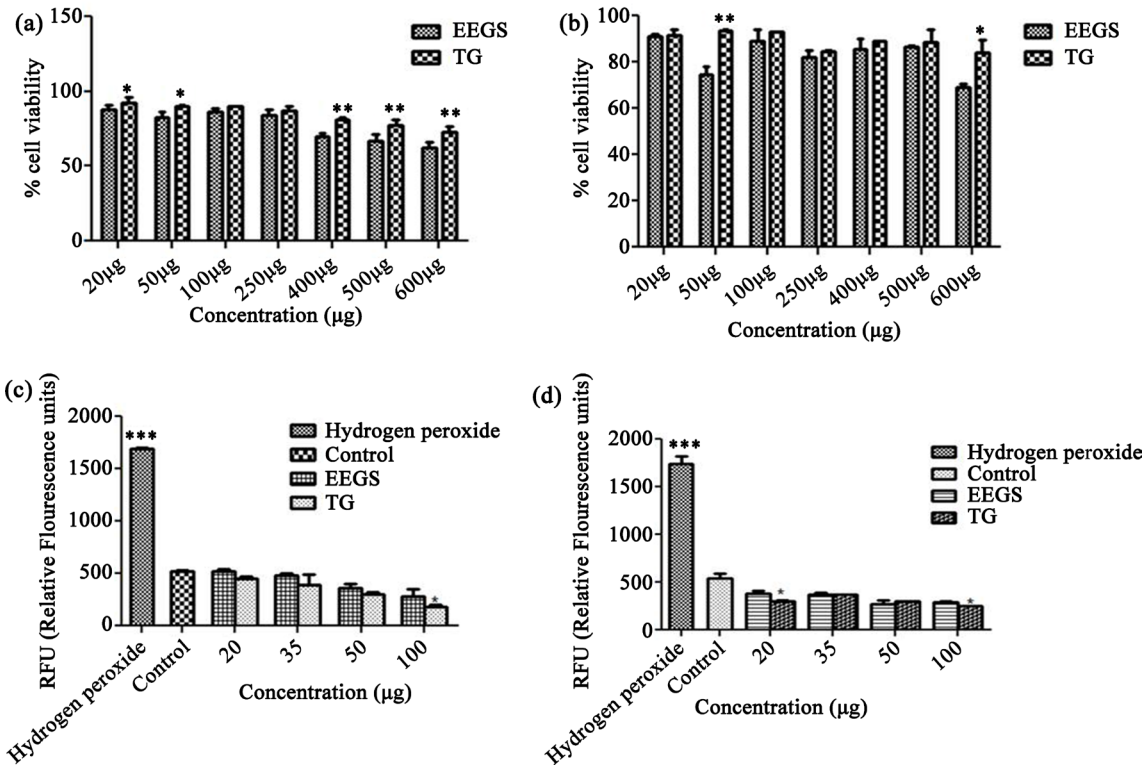
using Graph Pad Prism version 5.0. The experiments were carried out at significance levels; $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

In our previous experiments, we had isolated active fraction from the ethanolic extract of *Gymnema sylvestre* (GS) using preparative gradient HPLC method and termed it as TG (mixture of Triterpene glycosides). The HPLC purified active fraction TG, was characterized using TLC, FTIR, LC-MS and NMR and identified to be a mixture of; gymnemic acid I, IV, VII and gymnemagenin [10]. In this study antidiabetic activity of TG was explored using *in vitro* cell line models.

3.1. Cytotoxicity Studies

Cytotoxicity assay indicated that ethanolic extract of *Gymnema sylvestre* (EEGS) and active triterpene glycoside fraction (TG) did not compromise on the cell viability of C2C12 skeletal muscles cells and 3T3L1 adipocytes, even at higher concentration of 600 µg (**Figure 1(a)**, **Figure 1(b)**). With myocytes 70% ($p <$



*represents significance at ($p < 0.05$) **represents significance at ($p < 0.01$). ***represents significance at ($p < 0.001$).

Figure 1. Effect of TG and EEGS on C2C12 and 3T3L1 cell's viability. MTT was performed to study the cytotoxic effects of TG and EEGS. C2C12 and 3T3L1 cells were treated with different concentrations of EEGS, TG and the cell viabilty was studied. (a) represents the cell viability of C2C12 and (b) represents viability of C2C12 cells on exposure to EEGS and TG treatment. (c) and (d) shows the ROS effect of EEGS and the active fraction TG on C2C12 and 3T3L1 cells. Treatment was carried out at concentrations 20, 35, 50 and 100 µg levels. H_2O_2 was used as positive control. All values were represented as mean± SD. Experiments were performed in triplicates. Analysis was performed using Two-way ANOVA.

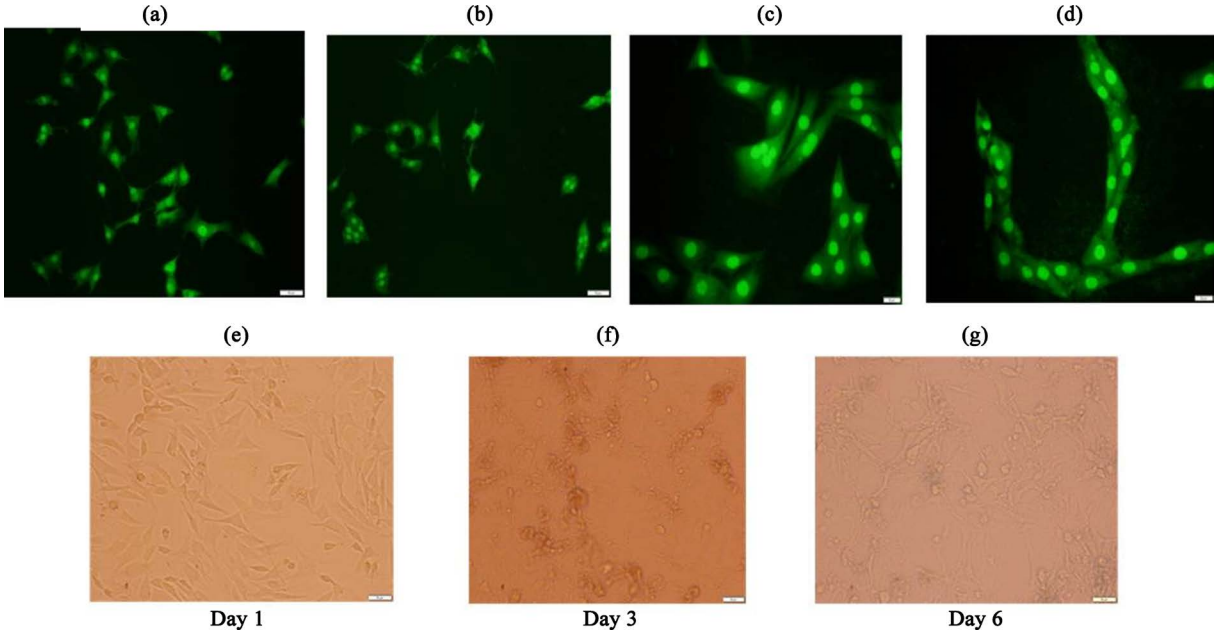
0.01) of cell viability was retained even at the highest concentration studied (600 µg) (Figure 1(a)). The 3T3L1 cells exhibited 90-95 % viability ($p < 0.05$) at 600 µg (Figure 1(b)). This demonstrated that TG and EEGS were non cytotoxic.

C2C12 and 3T3L1 cell were treated with TG and EEGS at 20, 35, 50 and 100 µg/ml concentrations to check the generation of oxygen species (ROS). H_2O_2 (200 µM) was used as positive control. A substantial drop ($p < 0.05$) in ROS generation was detected in both C2C12 and 3T3L1 cells compared to the positive control at the respective concentrations studied (Figure 1(c), Figure 1(d)). Nevertheless, the increase in sample concentration showed small incremental significance.

3.2. Cell Morphological Observation

To further observe the changes in C2C12 differentiation, the treated cells were stained using acridine orange (AO). The figures show clear changes in the morphology with a prominent nucleus and the spindle-shaped thick fibres (Figure 2(c), Figure 2(d)). Figure 2(a) & Figure 2(b) depict the undifferentiated myotubes.

When differentiation inducing medium was introduced, cells multiplied rapidly, and cell volume gradually became more massive, leading the cellular morphology to change from spindle-shape to oval. A tiny amount of small lipid droplets could be observed 1 day after induction (Figure 2(e)). Lipid droplets showed up in most



*represents significance at ($p < 0.05$). **represents significance at ($p < 0.01$). ***represents significance at ($p < 0.001$).

Figure 2. Morphological observation in C2C12 and 3T3L1 cells. The figure illustrates the morphological changes seen after differentiation in C2C12 myocytes after staining with a fluoroscent dye acridine orange (AO). (a) and (b) represents the undifferentiated cells, with a smaller nuclei and cells dispose almost star shape. (c) and (d) shows clear differentiation of cells, with a prominent nucleus, and cells attaining spindle shaped morphology. (e)-(g) shows the respective differentiating stages of 3T3L1 cells on Day 1, 3 and 6. The cells attain round grape bunch like morphology with accumulation of lipid droplets.

cells 3 days later (Figure 2(f)) and the differentiation rate reached more than 60%. As the cultivation time extended, small lipid droplets gathered into a grape-like bunches (Day 6) (Figure 2(g)).

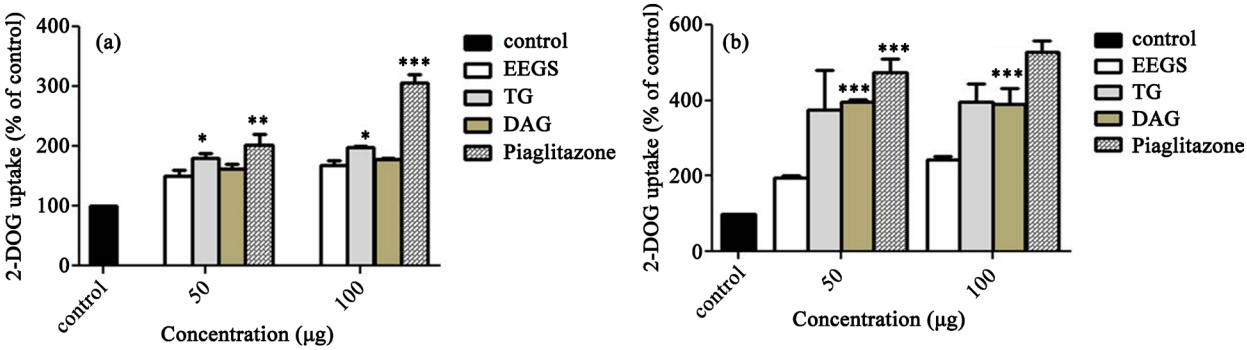
3.3. Glucose Uptake Assay

Glucose uptake activity of EEGS and active fraction TG were assessed using pioglitazone (50 µg/mL) as the reference standard. The active fraction TG and EEGS at concentrations, 50 and 100 µg/mL induced dose-dependent stimulation of glucose uptake in adipocytes as well as in skeletal muscle cells. Significantly, TG at 50 µg/mL concentration exhibited a better increase in glucose uptake compared to the control in both cell types (Figure 3(a), Figure 3(b)). In 3T3L1 cells, EEGS at 50 µg/mL concentration, increased glucose uptake by 0.5 times compared to the control and at 100 µg/mL EEGS level, the increase was 1 times compared to control. With TG, at 50 µg/mL level, increase in glucose uptake was 0.8 times than that of control, while at 100 µg/mL concentration, the increase was slightly better (1 fold) (Figure 3(a)).

However, in C2C12 cells, at 100 µg/mL, TG induced a momentous increase in glucose uptake up to 2.5 times more than that of control in C2C12 cells (Figure 3(b)). The cellular glucose uptake at 100 µg/mL was better with active fraction TG treatment than that with the crude extract EEGS (2.2 times more).

3.4. Effect of EEGS and TG on glut-4, ppar-γ, Adiponectin, and Leptin Transcription Level

Performance of the crude EEGS and active fraction TG on the mRNA expression levels of glut-4, ppar-γ, adiponectin, and leptin by Real-Time-Quantitative PCR were studied. There were elevated expressions after treatment compared to the control. It was observed that there was significant increase in Glut-4 transcription by 0.7 folds in 3T3L1 cells (Figure 4(a)) and 1.5 folds in C2C12 cells with TG treatment (100 µg/mL) compared to control cells ($p < 0.01$) (Figure 4(b)).



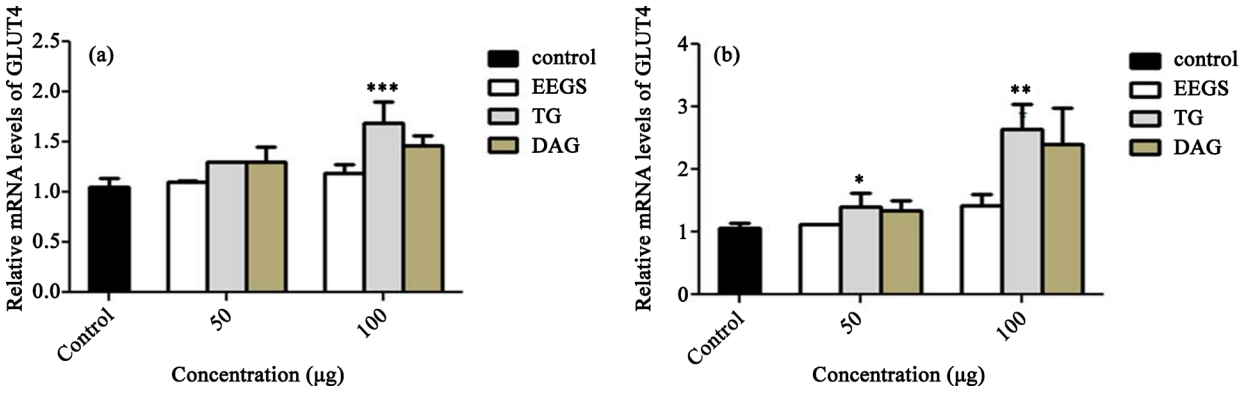
*represents significance at ($p < 0.05$). **represents significance at ($p < 0.01$). ***represents significance at ($p < 0.001$).

Figure 3. Effect of EEGS and TG on Glucose uptake. Both myocytes and adipocytes were treated with TG at 50 and 100 µg concentrations. Deacylgymnemic acid (DAG), an available commercial standard from GS and Pioglitazone, a commercial drug used for diabetes treatment were also used for comparison. (a) represents effect of TG on increased uptake of glucose in 3T3L1 cells and (b) represents effect of TG on increased uptake of glucose in C2C12 cells respectively. All values were represented as mean ± SD. Experiments were performed in triplicates. Analysis was performed using Two-way ANOVA.

The increase was comparatively better than commercial standard DAG (100 μg/mL). Though EEGS contributed to an increase in glut-4, the increase was less compared to the active fraction, TG.

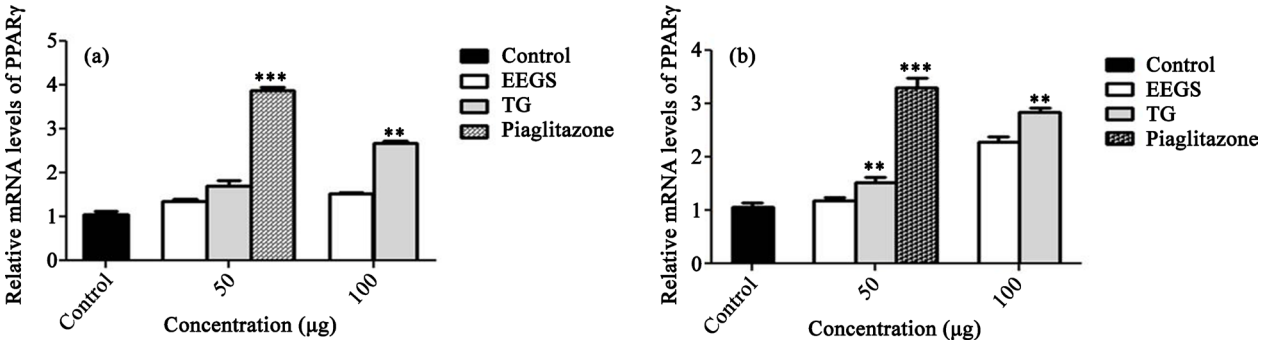
Further, role of ppar-γ in glucose transport as mediated by TG was analyzed. Up-regulation of ppar-γ transcription by TG, was increased by 1.5 fold in C2C12 cells (Figure 5(a)) and 0.5 folds in 3T3L1 cells (Figure 5(b)), respectively, relative to control cells at 50 μg/mL of TG ($p < 0.01$). While pioglitazone exhibited 3.0 fold increase in C2C12 and 2.2 folds in 3T3L1 cells at 50 μg/mL (Figure 5(a), Figure 5(b)), while EEGS-treated cells slightly altered the ppar-γ expression in both cell types which was 0.5 folds 50 μg/mL (Figure 4(c)). At 100 μg/mL of TG, there was 1.5 fold increase in ppar-γ in C2C12 cells and 2.0 folds increase in 3T3L1 cells, however with EEGS, the increase was 1 fold in 3T3L1 cells. (Figure 5(a), Figure 5(b)).

Furthermore, TG showed significant ($p < 0.001$) enhanced expression of adiponectin by 1.5 folds at 50 μg/mL (Figure 6(b)) and EEGS enhancement was almost equal to control at the same concentration. The standard drug induced



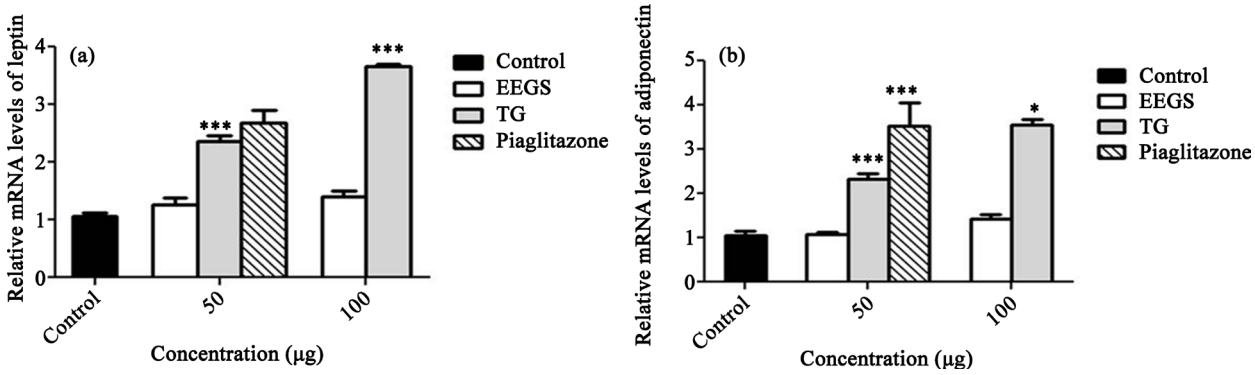
**represents significance at ($p < 0.01$). ***represents significance at ($p < 0.001$).

Figure 4. Effect of EEGS and TG on mRNA levels of GLUT-4. The figure represents effect of TG and EEGS on increased levels of GLUT-4 in C2C12 (Figure 4(a)) and 3T3L1 cells (Figure 4(b)) respectively. Experiments were performed in triplicates. Analysis was performed using Two-way ANOVA.



**represents significance at ($p < 0.01$). ***represents significance at ($p < 0.001$).

Figure 5. Effect of EEGS and TG on mRNA levels of PPAR-γ. The figure represents effect of TG on increased levels of GLUT-4 in C2C12 (Figure 5(a)) and 3T3L1 cells (Figure 5(b)) respectively. Experiments were performed in triplicates. Analysis was performed using Two-way ANOVA.



*represents significance at ($p < 0.05$). **represents significance at ($p < 0.01$). *** represents significance at ($p < 0.001$).

Figure 6. Effect of EEGS and TG on mRNA levels of adiponectin and leptin. The figure represents effect of TG on increased levels of adiponectin (a) and leptin (b) 3T3L1 cells respectively. Experiments were performed in triplicates. Analysis was performed using Two-way ANOVA.

adiponectin expression almost equal to TG. At 100 µg/mL level there was 1.5 fold increase in adiponectin levels with EEGS and 2.5 fold increase with TG. At 50 µg/mL there was 1.0 fold increase at in expression of leptin by TG relative to control. However, at 100 µg/mL, the increase was 2.5 folds (**Figure 6(a)**). At 50 µg/mL concentration of TG, the enhancement was almost in par with pioglitazone.

4. Discussion

Type 2 Diabetes is a metabolic disorder with diverse biochemical entities which are involved in the development of altered glucose transport related with defective GLUT-4 translocation and impaired insulin signalling cascade [23]. The declined GLUT-4 translocation and defective insulin signalling cascade were demonstrated as one among the predominant factors in insulin resistance [23]. Hence, there is a need for discovery of safe phytoactive agents that enhance the GLUT-4 expression and further enhance the cellular glucose uptake. In this context, C2C12 and 3T3L1 adipocyte cell lines are reported to be very good to be studied as *in vitro* models to explore GLUT activity. Moreover, skeletal muscle is the prime site for primary glucose clearance and glucose utilisation [24]. Hence these two cell lines were selected for our study.

Negligible to no toxicity is crucial for the effective development of a pytotherapeutic preparation [25]. In the current study, the crude extract EEGS and the active fraction of *Gymnema sylvestre* (TG) were screened for cytotoxicity against 3T3L1 adipose and C2C12 muscle cells at different concentrations. At highest concentration studied (600 µg/ml), the active fraction showed maximum cell viability around 90% - 95% in adipocytes and 70% - 80% viability in myocytes. Hyperglycemia-induced impairments in redox balance are considered a key trigger of diabetic complications, through the up-regulated generation of reactive oxygen species (ROS), together with an impaired ability of the endogenous antioxidant defence system to remove them [26]. In one of the *in vitro* studies, *Gymnema sylvestre* alcoholic leaf extract showed antioxidant ability by inhibit-

ing 1,1-diphenyl-2-picrylhydrazyl (DPPH) and scavenging superoxide and hydrogen peroxide [26]. In the current study, H_2O_2 was chosen as known model biological ROS. A significant reduction in the formation of ROS was observed compared to control at all the concentrations of TG studied.

Previous reports showed that L6 myotubes displayed maximum glucose uptake activity by troglitazone and rosiglitazone at 10 and 100 μM concentrations, respectively [13]. In one more study, a significant dose-dependent upsurge was observed in glucose uptake in all the tested concentrations of methanolic extract of *Gymnema sylvestre* (MLGS). Additionally, the glucose uptake activity of MLGS (750 μg/ml), was in par with rosiglitazone (50 μg/ml) [13]. Also, the greater glucose uptake in L6 cells was majorly intervened through increased GLUT-4 level [13]. Thus, the findings in our study comply with the literature reports where there is a concomitant increase in glucose uptake along with enhanced GLUT-4 levels in C2C12 muscle cells. Similar results were obtained with 3T3L1 adipocytes. Altogether, the above findings evidenced that glucose uptake is dependent on increased or decreased expression of GLUT-4. Further, in our study enhanced glucose uptake was concomitant with the increased expression of GLUT-4 encoding mRNA, in adipocytes and myotubes on incubation with TG [27].

Similarly, PPAR-γ, a nuclear transcriptional factor known for its critical role in the insulin receptor signalling cascade in enhancing glucose uptake. The levels of PPAR-γ are noted to be lower in insulin resistance. The PPAR-γ agonists belonging to the chemical class of thiazolidinedione's (TDZs) (such as rosiglitazone and pioglitazone) play a crucial role in reversing the insulin resistance [28]. Further, PPAR-γ activation leads to enhanced glucose-stimulated insulin secretion and increased insulin sensitivity [29]. Interestingly, elevated levels of PPAR-γ transcript by the active fraction TG correlated with enhanced GLUT-4 transcription and glucose uptake. So these observations confirmed that the increased glucose uptake, and GLUT-4 transcription were indeed due to the activation of PPAR-γ by TG, acting like PPAR-γ agonist [30].

Additionally, adiponectin is an adipokine that is profusely expressed and put forth a strong insulin-sensitising effect through binding to its receptors AdipoR1 and AdipoR2, through other unknown signalling pathways. Thus, levels of adiponectin in serum have a positive correlation with insulin sensitivity [31]. Adiponectin improves insulin sensitivity by three primary mechanisms. In skeletal muscle adiponectin increases the expression of CD36 and acyl-coenzyme-A oxidase molecules involved in transport and combustion of FA, and also increases uncoupling protein 2 required during energy dissipation. These changes decrease triglyceride content in skeletal muscle, which contributes to improved insulin signalling transduction [32]. Furthermore, adiponectin stimulates phosphorylation of acetyl coenzyme-A carboxylase, increase fatty-acid combustion, glucose uptake, and lactate production in myocytes. These changes reduce gluconeogenesis in the liver; thus, adiponectin shows acute glucose lowering effect [32]. Remarkably, the PPAR-γ agonist belongs to thiazolidinedione's (TZDs) class are known to elevate the circulating adiponectin levels, this is well estab-

lished by exposing 3T3L1 cells to thiazolidinedione's (TZDs) [33]. In earlier reports, methanolic extract of *Gymnema sylvestre* (MLGS) showed enhanced expression of adiponectin, thus confirming the ameliorative effect of MLGS on insulin resistance [22].

In support of above reports, in the current study, the effect of EEGS and active fraction TG on adiponectin levels was assessed using 3T3-L1 cell lines. There was a substantial upsurge in expression of adiponectin in TG and pioglitazone-treated groups. Apart from adiponectin, Leptin, another important adipocytokine majorly secreted by adipocytes, inhibits appetite, stimulates thermogenesis, decreases blood glucose, and reduce body weight and fat. Leptin also directly affects glucose metabolism in the liver and interferes with lipid metabolism [34]. The previous records from *in vitro* and in vivo studies suggested that leptin promoted energy dissipation and decreased lipid deposition in adipose tissues [34]. Altogether, expression of leptin exhibits profound positive relation with the glucose metabolism and insulin signalling. Earlier, the effect of methanolic extract of *Gymnema sylvestre* (MLGS) was noted on leptin expression in 3T3-L1 cells using rosiglitazone as the reference standard. A significant increase in leptin levels was observed in MLGS as well as rosiglitazone-treated groups [22]. In the same way, even in our studies, we observed a significant increase in leptin levels in 3T3-L1 cells *in vitro* in relation to pioglitazone as the reference standard. The leptin levels at 50 μg/ml concentration of TG was almost in par with the reference standard.

These findings indicated that there has been GLUT-4, PPAR-*γ*, adiponectin and leptin gene expressions to link with the antidiabetic activity of TG. The role of GLUT-4, PPAR-*γ*, adiponectin, and leptin in studying insulin resistance is well substantiated with available scientific literature. Thus, these factors have a crucial role in etiology of diabetes and related complications. Our findings indicated that TG could dose-dependently enhance the expression of GLUT-4, PPAR-*γ*, adiponectin, and leptin, and thereby exhibiting potential antidiabetic activity.

5. Conclusion

The present study revealed that the active triterpene glycoside fraction (TG) enhanced the glucose uptake efficiency of 3T3-L1 adipocytes and C2C12 myocytes, thus signifying to be glucose lowering agent in the management of diabetes. The part of markers GLUT-4, PPAR-*γ*, adiponectin, and leptin in ameliorating insulin resistance is well approved with existing literature reports. Thus, these factors have a crucial role in etiology of diabetes and related complications. In the current study, an effort was made to explore the mechanism involved in the antidiabetic activity of active fraction of *Gymnema sylvestre* (GS). The outcomes of the study indicated that TG could dose-dependently augment the expression of the glucose sensitive markers; GLUT-4, PPAR-*γ*, leptin and adiponectin, thus owing to its possible antidiabetic activity. These outcomes suggest that the processed plant's active fractions/compounds may be used as a functional phytoin-

gredient/nutraceutical candidate in the treatment as well as management of diabetes.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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