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Glucose uptake and Alpha-Amylase Inhibitory Activity of *Gymnema sylvestre* using Pancreatic (RIN-m5F) and Adipocyte (3T3-L1) Cell Lines

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Abstract: The present study was designed to investigate the antidiabetic activity of *Gymnema sylvestre* (GS) in an in vitro analysis of the ethanol extracts using RIN-m5F pancreatic and 3T3-L1 adipocyte cell lines. 3T3-L1 and RIN-m5F cell lines were procured from NCCS, Pune, and cultured in DMEM (high glucose) supplemented with 10% FBS, 10,000 units of Penicillin G, and 10,000 µg/ml streptomycin sulfate and 10 mM HEPES. The cultures were maintained at 37°C with 5% CO_2 in a humidified incubator. The ethanolic extract of GS induced cytotoxic effects on RIN-m5F pancreatic and 3T3-L1 adipocyte cell lines, and an MTT assay was performed. When extracts were used at concentrations ranging from 0.039 to 5 mg/ml, the viability was retained up to 70% ($p<0.05$). In the ethanolic extract of GS at all concentrations from 5 mg/ml to 0.039 mg/ml, the glucose uptake level was higher when compared to the control. The percentage of alpha-amylase inhibition was obtained after GS treatment at different concentrations. GS at 1000 µg/ml showed a higher percentage of inhibition (60.17%). The RIN-m5F cell line offers important models to study the mechanisms of insulin and somatostatin secretion mechanisms, identify hormone receptors and antigens on their surfaces, and determine the nutritional requirements for hormone secretion in a defined medium. The 3T3-L1 adipocyte cell line is for glucose uptake in peripheral tissues such as adipose tissue, which is considered an important step in regulating glucose homeostasis.

Keywords: *Gymnema sylvestre*, Antidiabetic activity, 3T3-L1 adipocytes cell lines, RIN -m5F pancreatic cell line, Glucose uptake, Alpha-amylase inhibitory activity

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Introduction

Diabetes Mellitus (DM) is an endocrine disorder that has a major impact on the health of the population, affecting millions of individuals across the world. The World Health Organisation estimates that worldwide hyperglycemia is the third leading cause of premature death. The International Diabetes Federation shortlisted India as one of the most afflicted countries with

the highest number of people suffering from diabetes in the Southeast Asia region. It was found that 69.1 million adults living in India suffer from diabetes. It is predicted that one of the largest absolute increases in the diabetic population is expected to occur in India, with the International Diabetes Federation estimating that India alone will have 100 million people with diabetes by 2030. In 2021, 537 million cases of diabetes were reported worldwide and are likely to increase to 783 million by 2045 (Hoogeveen, 2022).

Gymnema sylvestre (GS) is a large woody climber plant from the Asclepiadaceae family and is native to central and southern India, tropical Africa, and Australia. This plant has been used as an Ayurvedic medicine for the treatment of diabetes in India for centuries (Kanetkar *et al.,* 2007). This herb is the original sugar destroyer. *Gymnema sylvestre* stimulates the pancreatic beta cells to produce more insulin in type II diabetes and enhances the ability of insulin to decrease blood glucose uptake and utilization by peripheral tissue (Baskaran *et al*., 1990). It may also decrease cholesterol and glucose absorption from the gastrointestinal tract (Shimizu *et al.,* 1997). Gymnemic acid is a class of chemical compounds isolated from the leaves of the plant *Gymnema sylvestre*, also known as the Australian cow plant. Gymnemic acid was first isolated in 1888. These compounds interact with taste receptors on the tongue to temporarily suppress the taste of sweetness (Sanematsu *et al*., 2014). Hence, the *Gymnema sylvestre* may have chemopreventive, anti-cancer, anti-microbial activity, antioxidant, anti-diabetic activity, anti-inflammatory, and antifungal due to the presence of secondary metabolites in the ethanolic extract (Sindhuja and Mary Agnes, 2023a).

Gymnema sylvestre has antioxidant, anti-inflammatory, antibiotic, anti-viral, hepato-protective, anti-cancer, and lipid-lowering activities (Khan *et al*., 2019). Gymnemic acid is considered to be the primary compound responsible for the anti-diabetic effects of *Gymnema sylvestre* (Tiwari *et al*., 2014).

Gymnemic acid has been extensively studied in models of hyperglycemia. The most common outcome across these various model systems is the ability of gymnemic acid, regardless of the route of administration, to reduce plasma glucose levels. Increased insulin secretion may modulate these glucose-lowering effects, as seen in *in vitro* experiments using insulinoma cell lines and islets (Al-Romaiyan *et al*., 2012). Insulin plays a central role in regulating blood glucose. In addition to the direct effects of hyperglycemia in enhancing glucose uptake into both the liver and peripheral tissues, the hormone insulin plays a central role in regulating glucose uptake levels as a direct response to the degree of hyperglycemia. The islet is freely permeable to glucose via this GLUT 2 transporter, and the high glucokinase phosphor-rylates the glucose. Therefore, the treatment of diabetes becomes indispensable. Many medications through synthetic drug treatment have been developed so far and are available for diabetic patients, but there is no report of total recovery from diabetes yet. Furthermore, those current oral hypoglycemic drugs also cause unwanted side effects. Thus, a shift towards alternative medicines, i.e., using different plant and herbal formulations, is needed (Khosla *et al*., 2000). Understanding the importance of traditional herbs becomes important as traditional medicines have a bright future in treating diabetes. Traditional medicines based on plants are affordable and easily accessible to 80% of people in developing nations. In treating and preventing diabetes, the ideal drugs should augment insulin secretion in pancreatic cells and glucose uptake in insulin-sensitive tissues. Multi-target therapy to control the various factors contributing to the development of diabetes will also support herbal medicine as an ideal drug. Therefore, this study targeted to evaluate cell line cytotoxicity, alpha inhibitory activity, and glucose uptake activity of the plant *Gymnema sylvestre* in 3T3-L1 adipocytes and RIN-m5F pancreatic cells (Persaud *et al*., 1999).

The 3T3-L1 adipocyte is one of the targets of insulin action, and adipose tissue plays an

important role in maintaining whole-body energy homeostasis. The 3T3-L1 cells are routinely used in signalling studies and insulin secretion. In several β-cell lines (RIN-m5F), treatment with an extract of *Gymnema sylvestre,* which contains gymnemic acid, at 0.125, 0.25, and 0.5 mg/ml for 1 h increased basal insulin secretion in a dose-dependent manner (Slater and Sawyer,1971). However, the ability of *Gymnema sylvestre* to trigger insulin release is the result of increased membrane permeability. The high saponins and glycoside content found in gymnemic acid increase plasma membrane permeability, resulting in insulin leakage from the cell rather than regulated exocytosis (Hassan *et al*., 2010).

Materials and Methods

Chemical and reagents

Following chemicals were purchased locally--90% Dubelocco's modified Eagles medium, 10% Fetal Bovine Serum, 90% DMEM, 10% Fetal Bovine Serum, 1 µM Dexamethane, 0.5 mM isobutyl methylxanthine, 1 µg/ml insulin, 90% DMEM, 10% Fetal bovine, Serum (FBS), 1 µg/ml insulin, 2-deoxyglucose (2-DG), 500 µl of phosphate buffer, 500 µl of 0.2% starch, 1% sodium chloride solution, 1 ml of DNSA (1 g of 3,5 dinitro salicylic acid), 30 g of sodium potassium tartrate, 20 ml of 2N sodium hydroxide. Ethanol has the nature of breaking down the phytochemical compounds of plant material (Sindhuja *et al*., 2020)

Plant extract

Fresh leaves of *Gymnema sylvestre* were collected from Vellore District, Tamil Nadu, India. Plant material was shade-dried and ground into a powder. The extract was prepared by successive maceration of the powder (1 g) at room temperature with an ethanol extract (10 ml) in a shaker for 24 h. The final extract was filtered, and this was used for the following assays.

Cell culture

3T3-L1 and RIN-m5F cell line was procured from NCCS, Pune, India and cultured in DMEM (high glucose) supplemented with 10% FBS, 10,000 units of Penicillin G, and 10,000 µg/ml streptomycin sulfate and 10 mM HEPES. The cultures were maintained at 37°C with 5% CO_2 in a humidified incubator.

In vitro Anti-diabetic activity Using 3T3 Cell line

Cytotoxicity Assay

The colorimetric MTT assay method is routinely used to assess cell viability. In the mitochondria of live cells, yellow MTT (3-(4, 5-Dimethlthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) is converted to purple formazan. Because this reduction occurs only when mitochondrial reductase enzymes are functioning, conversion is directly proportional to the amount of viable (live) cells. The cells were seeded in 96 well plates and then treated for 24 h with varied concentrations of the extracts (100 ng, 10 ng, 1 ng, 1 g, 10 g, and 100 g). Cells were treated with MTT reagent for 4 h after incubation. After aspirating the MTT reagent, the produced formazan crystals were dissolved in DMSO and the absorbance at 595 nm was measured, which is directly proportional to cell viability (Tables 1, 2). The percentage of cytotoxicity demonstrated by the extracts was estimated based on cell viability, and the results showed statistical significance (Khan *et al*., 2019).

Glucose uptake measurement Using 3T3-L1 Cell Line

3T3-L1 pre-adipocyte cells were seeded and maintained with pre-adipocyte expansion medium (PEM) for 48 h before incubating at 37°C and 5% CO_2, or to reach 100% confluence. The identical volume of medium was replaced with differen-tiation medium (DM) and incubated for 48 h at 37% in a humidified atmosphere containing 5% CO_2 with fruit extract for 6 days and transferred to adipocyte maintaining medium (AMM) and fraction was changed every 48 h of the experiment period. 2-deoxyglucose (2-DG) was used in glucose uptake assay protocols because of its structural similarity to glucose. 2-DG is taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P). 2-DG6P can not be further metabolized and thus accumulates within cells.

Table 1: 3T3L1 adipocyte: Optical Density, Calculated Mean Value, and % Viability of Negative Control, Positive Control

ID	Conc. (mg/ml)	OD I	OD II	Mean OD	SD	% CV	% Viability
Blank	NA	0.0538	0.0531	0.05345	0.0005	0.9261	NA
NC	NA	0.1189	0.1197	0.1193	0.0006	0.4742	100
PC	5	0.0618	0.0611	0.06145	0.0005	0.8055	51.51
TC1	5	0.0857	0.0862	0.08595	0.0004	0.4113	72.05
TC2	2.5	0.0888	0.0905	0.08965	0.0012	1.3409	75.15
TC3	1.25	0.0919	0.0922	0.09205	0.0002	0.2305	77.16
TC4	0.625	0.0947	0.0949	0.0948	0.0001	0.1492	79.46
TC5	0.312	0.0961	0.0972	0.09665	0.0008	0.8048	81.01
TC6	0.156	0.099	0.1005	0.09975	0.0011	1.0633	83.61
TC7	0.078	0.1018	0.1014	0.1016	0.0003	0.2784	85.16
TC8	0.039	0.1029	0.1035	0.1032	0.0004	0.4111	86.50

NC – Negative control , PC – Positive Control , TC – Test Control

Table 2: RIN-m5F Pancreatic: Optical Density, Calculated Mean Value, and % Viability of Negative Control, Positive Control

ID	Conc. (mg/ml)	OD I	OD II	Mean OD	SD	% CV	% Viability
Blank	NA	0.0587	0.0589	0.0588	0.0001	0.2405	NA
NC	NA	0.115	0.1158	0.1154	0.0006	0.4902	100
PC	5	0.0598	0.0578	0.0588	0.0014	2.4051	50.95
TC1	5	0.0811	0.0815	0.0813	0.0003	0.3479	70.45
TC2	2.5	0.0888	0.0905	0.08965	0.0012	1.3409	77.69
TC3	1.25	0.0919	0.0922	0.09205	0.0002	0.2305	79.77
TC4	0.625	0.0929	0.0936	0.09325	0.0005	0.5308	80.81
TC5	0.312	0.0955	0.0949	0.0952	0.0004	0.4457	82.50
TC6	0.156	0.098	0.0989	0.09845	0.0006	0.6464	85.31
TC7	0.078	0.101	0.1015	0.10125	0.0004	0.3492	87.74
TC8	0.039	0.1029	0.1033	0.1031	0.0003	0.2743	89.34

NC – Negative control , PC – Positive Control , TC – Test Control

The accumulated 2-DG6P is directly proportional to 2-DG (or glucose) uptake by cells. After 24 h of incubation, 1 M 2-deoxyglucose (2-DG) was added to the cells and cells treated with 100 µl of test item at eight concentrations (5 mg/ml). Insulin (1 IU), negative control (cell alone) and blank (media only) were added in duplicates into the respective wells of the 96-well plate. The cells were further incubated for 0.5 h at 5% CO_2 at 37°C with >90% humidity and the glucose uptake process was

terminated by adding an ice-cold buffer solution. The content in each well was transferred to the individually labeled 2ml centrifuge vials. The contents were centrifuged at 1100 rpm for 5 min. Then the cell culture supernates were collected in fresh labeled Eppendorf tubes. The glucose utilized in the cell was measured using Standard Glucose commercial kit, Biosystem. The enzyme activity was directly proportional to the available glucose which was measured. To 10 µl of supernatant, 1 ml of glucose reagent was added and the absorbance was read at 405 nm using a multi-plate reader.1 ml of glucose reagent alone served as Blank. The experiments were done in duplicate. The percentage of Glucose uptake in the test sample was calculated from a control sample. The assay samples were kept at 2-8°C till analysis.

Alpha-amylase inhibitory assay Using 3T3-L1 Cell line

This assay was performed as per DNSA Miller GL. method. 500 µl of phosphate buffer, 500 µl of 0.2% starch, and 1% sodium chloride solution were pre-incubated with the test sample 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390 and 0.195) and control sample for 5 min, followed by 200 µl of porcine pancreatic amylase added and the mixture was incubated at 37ºc for 15 min. The reaction was stopped by adding 1 ml of DNSA (1 g of 3,5 dinitro salicylic acid, 30 g of sodium potassium tartrate, and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and it was kept it in a boiling water bath for 1 min. Then the reaction mixture was diluted with 2.2 ml of water and absorbance was read at 540 nm. The percentage of inhibition of the test sample was calculated from a control sample.

In vitro Anti-diabetic activity Using RIN-m5F Cell line

Cytotoxicity test

RIN-m5F pancreatic cells were seeded into 200 µl 96-well plates at a density of 6.0×10^3 cells per well**.** The cells were allowed to attach for 48 h, Cells were lysate with 1% Triton X 100 for 10 min. after which they were treated for 24 h at 37 °C with 100 µl of plant extract at varying concentrations **(**0.625, 1.25, 2.5, 5.0, and 10.0 g/100 L*), added to the designated wells.

Following treatment, 50 µl of MTT solution (5 mg ml^{-1}) was added to each well (both treated and untreated controls), and the plates were incubated in the dark at 37 °C for 3 h**.** After incubation, the culture medium was carefully removed from each well, and 200 µl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals (Tables 3, 4).

The optical density (OD) was measured at 540 nm using a microplate reader (ECIL Micro Scan MS5608A).

Glucose uptake measurement Using RIN-m5F Cell Line

2-deoxyglucose (2-DG) was used in glucose uptake assay protocols because of its structural similarity to glucose. 2-DG is taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P). 2-DG6P can not be further metabolized and thus accumulates within cells. The accumulated 2-DG6P is directly proportional to 2-DG (or glucose) uptake by cells. After 24 h of incubation, 1 M 2-deoxyglucose (2-DG) was added to the cells and cells treated with 100 µl of test item at eight concentrations (5 mg/ml), Insulin (1 IU), negative control (cell alone) and Blank (media only) were added in duplicates into the respective wells of the 96-well plate. The cells were further incubated for 0.5 h at 5% CO_2 at 37°C with >90% humidity and the glucose uptake process was terminated by adding an ice-cold buffer solution. Cells were lysated with 1% Triton X 100 for 10 min. The content in each well was transferred to the individually labeled 2 ml centrifuge vials. The contents were centrifuged at 1100 rpm for 5 min. Then the cell culture supernates were collected in fresh labeled Eppendorf tubes. The glucose utilized in the cell was measured using Standard Glucose commercial kit, Biosystem. The enzyme activity was directly proportional to the available

Table 3: Plate Mapping for Cell Seeding and Exposure for Experiments (3T3-L1 adipocytes Cell lines)

	1	2	3	4	5	6	7	8	9	10	11
A	Blank	NC	PC	TC1-5 mg/ml	TC1-2.5 mg/ml	TC1-1.25 mg/ml	TC1-0.625 mg/ml	TC1 0.313 mg/ml	TC1-0.156 mg/ml	TC1-0.078 mg/ml	TC1-0.039 mg/ml
B	Blank	NC	PC	TC1-5 mg/ml	TC1-2.5 mg/ml	TC1-1.25 mg/ml	TC1-0.625 mg/ml	TC1 0.313 mg/ml	TC1-0.156 mg/ml	TC1-0.078 mg/ml	TC1-0.039 mg/ml

NC – Negative control , PC – Positive Control , TC – Test Control

Table 4: Plate Mapping for Cell Seeding and Exposure for Experiments (RIN-mf5 pancreatic Cell lines)

	1	2	3	4	5	6	7	8	9	10	11
A	Blank	NC	PC	TC1-5 mg/ml	TC1-2.5 mg/ml	TC1-1.25 mg/ml	TC1-0.625 mg/ml	TC1 0.313 mg/ml	TC1-0.156 mg/ml	TC1-0.078 mg/ml	TC1-0.039 mg/ml
B	Blank	NC	PC	TC1-5 mg/ml	TC1-2.5 mg/ml	TC1-1.25 mg/ml	TC1-0.625 mg/ml	TC1 0.313 mg/ml	TC1-0.156 mg/ml	TC1-0.078 mg/ml	TC1-0.039 mg/ml

NC – Negative Control, PC – Positive Control, TC – Test Control

glucose was measured. To 10 µl of supernatant, 1 ml of glucose reagent was added and the absorbance was read at 405 nm using a multi-plate reader (Board). 1ml of glucose reagent alone served as Blank. The experiments were done in duplicate. The percentage of Glucose uptake in the test sample was calculated from a control sample. The assay samples were kept at 2-8°C till analysis.

Alpha-amylase inhibitory assay Using RIN-m5F Cell line

This assay was performed as per the DNSA Miller GL method. 500 µl of phosphate buffer, 500 ml of 0.2% starch and 1% sodium chloride solution was pre-incubated with the test sample (100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390 and 0.195) and control sample for 5 min, followed by 200 µl of porcine pancreatic amylase was added and the mixture was incubated at 37 ºC for 15 min. The reaction was stopped by adding 1 ml of DNSA (1 g of 3,5 dinitro salicylic acid, 30 g of sodium potassium tartrate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and it was kept it in a boiling water bath for 1 min. Then the reaction mixture was diluted with 2.2 ml of water and absorbance was read at 540 nm. The percentage of inhibition of the test sample was calculated from a control sample.

Results

In vitro cytotoxicity assay

The ethanol extracts of *Gymnema sylvestre* did not affect the viability of both 3T3-L1 and RIN-m5F cells when compared to the control. It was observed that, when the highest concentration (100 µg/ml) of the extract was used in the cell culture, the viability of cells decreased, i.e. cell death was up to 0.039 mg/ml to 5 mg/ml (Tables 3, 4). Plant extract concentrations ranging from 1 mg/ml to 100 mg/ml exhibited very less toxicity, which means they do not involve in cell death, where the LC_{50} value is less than 50% even at higher concentrations in this study and hence could be considered as non-toxic and safe which might be suggested as a safe plant-based medicine to treat diabetes (Figs. 1, 2). In 3T3-L1 Cell Line percentage of cell viability of *Gymnema sylvestre* at 0.039 mg/ml, 0.078 mg/ml, 0.156 mg/ml, 0.312 mg/ml, 0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml and 5 mg/ml were 86.50, 85.16, 83.61, 81.01, 79.46, 77.16, 75.15 and 72.05, respectively (Fig. 1). No cytotoxicity was observed in negative control. A decrease in the amount of blue-violet formazan

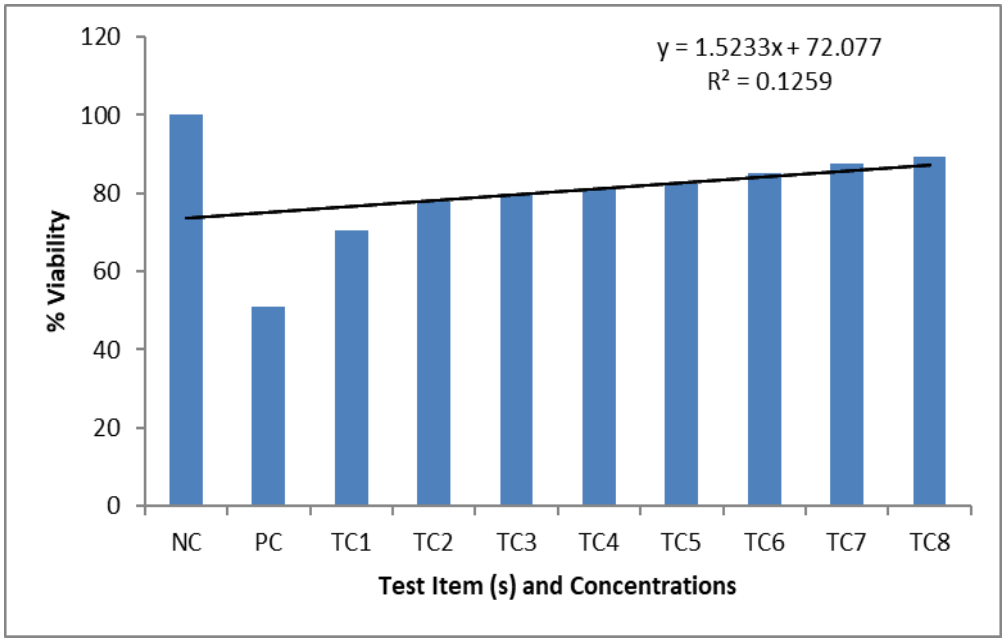

Fig. 1: Concentration Vs. % Viability for GS (RIN-m5F Cell line).

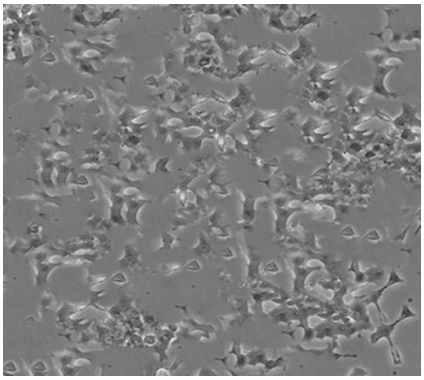

Fig. 2: Microscopical image of GS at 5mg/ml (RIN-m5F Cell Line).

was noted at 5 mg/ml, which confirms a decrease in the number of living cells. The viability was found to be lesser than 70% of the negative control. RIN-m5F Cell Line Percentage of viability of GS at 0.039 mg/ml, 0.078 mg/ml, 0.156 mg/ml, 0.312 mg/ml, 0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml, and 5 mg/ml were 89.34, 87.74, 85.31, 82.50, 80.81, 79.77, 77.69 and 70.45, respectively (Fig. 2).

The results of glucose uptake activity were equated with standard drugs like insulin *Gymnema sylvestre* at all concentrations from 5 mg/ml to 0.039 mg/ml, the level of glucose uptake was found to be more when compared to the control. Uptake was observed as follows: 20 mg/ml – 64.47%, 1.25 mg/ml – 58.91%, 0.313 mg/ml – 51.24%, 0.156 mg/ml – 44.97%, 0.078 mg/ml – 40.13%, and 0.039 mg/ml – 31.62% (Table 5). The maximum uptake was recorded at a concentration of 5 mg/ml for TC 4, showing 68.05%, which is comparable to the standard antidiabetic drug insulin (76.68%) (Fig. 3).

The optical density and percentage inhibition obtained for *Gymnema sylvestre* treated at different concentrations is shown in Figure 4. GS at 1000 µg/ml showed a higher percentage of inhibition (60.17%). Inhibition of α-amylase activity by *Gymnema sylvestre* extract was tested against the control drug acarbose. GS leaf extract inhibited α-amylase activity by 42.55% at 62.50

Table 5: Glucose concentration in GS-treated cells (RIN-m5f Cell line)

ID	mg/dl (1)	mg/dl (2)	Mean mg/dl	SD	%CV	% glucose uptake
NC	48	51	49.5	2.121	4.29	NA
PC (insulin)	179	177	178	1.414	0.79	76.68
TC1- 5 mg/ml	125	120	122.5	3.536	2.89	68.05
TC2- 2.5 mg/ml	110	99	104.5	7.778	7.44	64.47
TC3- 1.25 mg/ml	90	91	90.5	0.707	0.78	58.91
TC4- 0.625 mg/ml	85	84	84.5	0.707	0.84	56.92
TC5- 0.313 mg/ml	80	79	79.5	0.707	0.89	51.24
TC6- 0.156 mg/ml	75	70	72.5	3.536	4.88	44.97
TC7- 0.078 mg/ml	62	62	62	0.000	0.00	40.13
TC8- 0.039mg/ml	55	59	57	2.828	4.96	31.62

NC – Negative control , PC – Positive Control , TC – Test Control

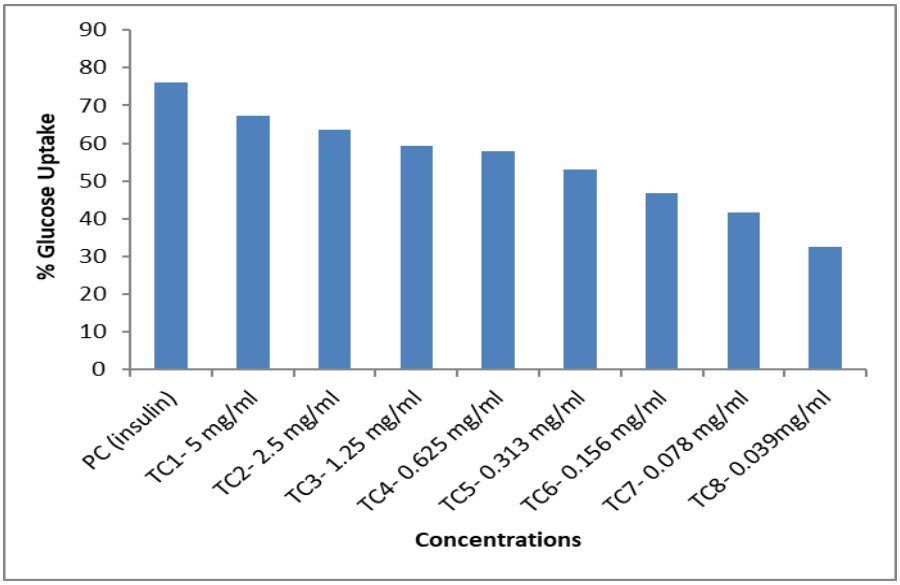

Fig. 3: Concentration Vs. Glucose uptake of GS (RIN-m5F Cell line).

µg/ml and 56.66% at 500 µg/ml concentration. The IC_{50} value of the extract was found to be 22.115 µg/ml. The control acarbose showed 37.15% inhibition of α-amylase activity at 31.25 µg/ml and 55.66% at 500 µg/ml concentration. The IC_{50} value of acarbose was found to be 51.29 µg/ml (Fig. 4).

Discussion

As a result, cytotoxicity tests must be performed before assessing the functional effects of plant extracts on mature adipocytes and their impact on cell viability and mitochondrial activity. The results showed that the *Gymnema sylvestre* extract

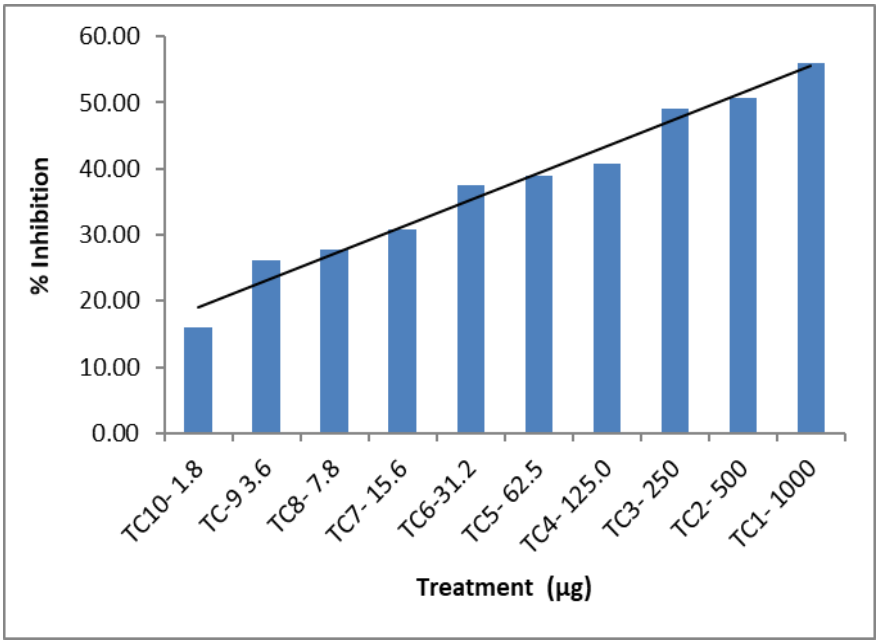

Fig. 4: Concentration Vs. % Inhibition of GS (RIN-m5F Cell line).

Table 6: Optical Density and Percentage Inhibition of GS-treated Cells (RIN-m5F Cell line)

Treatment/Control (µg)	OD 1	OD 2	Mean OD	SD	% CV	% Inhibition
Positive Control	0.2634	0.2696	0.2665	0.00	1.6	NA
TC10- 1.8	0.2468	0.2497	0.24825	0.00	0.8	6.85
TC-9 3.6	0.2117	0.2145	0.2131	0.00	0.9	20.04
TC8- 7.8	0.2084	0.2049	0.20665	0.00	1.2	22.46
TC7- 15.6	0.1894	0.189	0.1892	0.00	0.1	29.01
TC6-31.2	0.1663	0.1606	0.16345	0.00	2.5	38.67
TC5- 62.5	0.1525	0.1537	0.1531	0.00	0.6	42.55
TC4- 125.0	0.1432	0.1403	0.14175	0.00	1.4	46.81
TC3- 250	0.1294	0.123	0.1262	0.00	3.6	52.65
TC2- 500	0.1164	0.1146	0.1155	0.00	1.1	56.66
TC1- 1000	0.1056	0.1067	0.10615	0.00	0.7	60.17

NC – Negative control , PC – Positive Control , TC – Test Control

has no effect on the cells and may have very low cytotoxicity at IC_{50} (Balasubramanian *et al*., 2023).

To examine whether the ethanolic extract of *Gymnema sylvestre* and its active fraction (GS) induced any cytotoxic effects, an MTT assay was performed. When extracts were used at concentrations ranging from 0.039 to 5 mg/ml, the viability retained up to 70% ($p<0.05$), even at the highest concentration studied (5 mg/ml). This demonstrated the nontoxic effects of *Gymnema sylvestre*. No cytotoxicity was observed in the negative control. A decrease in the amount of blue-violet formazan was noted at 5 mg/ml, which confirms a decrease in the number of living cells.

The viability was found to be lesser than 70% of the negative control. *Gymnema sylvestre* concentrations from 0.039 to 5 mg/ml were found to be non-cytotoxic in terms of viability. According to the MTT experiment, dead cells and their products do not convert tetrazolium to create formazan crystals. Only active cells' enzymatic reactions decrease tetrazolium salts. Therefore, MTT assay can be converted to a blue formazan by the mitochondrial enzyme succinate dehydrogenase. The amount of formazan generated is proportional to the number of activated cells (Nirmala *et al*., 2018).

There is statistically no significant difference between the treatment and control groups. The plant extract values were nearly equivalent to the control groups. Glucose uptake in adipose tissue is one of the critical factors for reducing postprandial blood glucose concentration and insulin was found to have a stimulatory function. Insulin also induced glucose uptake by increasing functional glucose transport molecules in the plasma membrane. Insulin-secreting property as secretagogues was also reported for several plant extracts which might also help in showing antidiabetic activity (Dachani *et al*., 2012). The transport of glucose in skeletal muscle can also be stimulated by contractile activity. Medicinal plants augment the glucose uptake by GLUT4 translocation and it was earlier reported by *in vitro* glucose model (Tiwari *et al*., 2016).

The major enzymes that metabolize carbohydrates in the small intestine is pancreatic *α*-amylase enzymes, which convert polysaccharides to monosaccharides. Antidiabetic drugs reduce the activity of these enzymes and regulate the postprandial blood glucose level in type 2 diabetic patients (Kuppusamy *et al*., 2011). The mechanisms of control of glucose levels vary with the drug. Some drugs stimulate insulin secretion or synthesis by the pancreatic beta-cells, others help in regenerating damaged pancreatic beta-cells, still, others increase insulin sensitivity triggering glucose uptake by fat and muscle cells, and certain others modify the activity of enzymes pertinent to glucose metabolism to reduce the absorption of sugars from the gut (Mall *et al*., 2009).

Gymnema sylvestre leaves ethanol extract activity is concentration-dependent in the *in vitro* assay. *Gymnema sylvestre* leaves extract significantly inhibited alpha-amylase (30.20%) at low concentration while at the higher concentration inhibition was 84.11% as compared to a standard drug. The highest concentration is nearest to the standard. In glucose uptake assay higher concentration was absorbed when compared to positive control. *Gymnema sylvestre* has played a vital role in inhibiting adipose tissue in order to reduce hyperglycemia, reported to possess significant anti-diabetic activity and hypolipidemic activity in alloxan-induced and normal fasting rats and the insulin-producing rat cell line RIN-m5F (Bhathena *et al*., 1982). In RIN-m5F cells, GLP-1 (Glucagon-like peptide-1, a potent incretin hormone secreted from the distal gut) increased the capacity and affinity of insulin binding in a time- and concentration-dependent manner (Drissi *et al*., 2021). *In vitro* evaluation of various synthetic compounds has demonstrated glucose-dependent intracellular Ca^{2+} accumulation, resulting in enhanced insulin secretion. They showed the hypoglycemic properties of *Gymnema sylvestre.* Cellular uptake of glucose from blood plays a crucial role in the reduction of DM. This is generally mediated by GLUT4 in the cell. On stimulation with antidiabetic drugs, GLUT4 is translocated from their intracellular sites to the cell surface. Insulin induces the translocation of GLUT4 through the phosphatidylinositol-3-kinase pathway (Benomar *et al*., 2006). PKB/AKT-mediated stimulation of glucose transport by insulin has also been reported in 3T3-L1 rat adipocytes. *Gymnema sylvestre* is traditional medicinal herb used to cure various diseases (Sindhuja *et al*., 2023b)

In this study, we investigated the antidiabetic properties of *Gymnema sylvestre*. Our results showed that the ethanolic extract of GS effectively inhibited α-amylase and glucose uptake activities.

The inhibitory effects were estimated with acarbose as the standard drug. Furthermore, *Gymnema sylvestre* had no cytotoxic effects on the cells. The implications of this study corroborate the previous studies on the inhibitory effect of other natural compounds of α-amylase and α-glucosidase activities. *Gymnema sylvestre* plants used in this study are of Indian origin and are well-known by herbal pharmacologists for their medicinal properties. This study provides scientific evidence of their anti-diabetic effect. These plants are very affordable to the common man and can be easily incorporated into their daily diets. They can be further analyzed to develop anti-diabetic drugs free from harmful side effects.

Conclusion

The ethanolic extract of *Gymnema sylvestre* inhibited the activity of the enzyme α-amylase and enhanced glucose uptake in 3T3-L1 and RIN-m5F cell lines. These results were comparable to the control. Further, the extract had no cytotoxic effect on the cells. In conclusion, *Gymnema sylvestre* extract has potential antidiabetic applications. Hyperglycemia and other acute and long-term complications are the results of partial or total insulin deficiency, which is the primary cause of diabetes mellitus, a chronic disorder. *Gymnema sylvestre* stimulates the pancreatic beta cells to produce more insulin in type II diabetes and enhances the ability of insulin to decrease blood glucose uptake and utilization by peripheral tissue.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, Auxilium College (Autonomous), Vellore, India.

Author Contributions

Each author has contributed equally in designing the research, conducting thorough analysis, drafting the manuscript, and refining its content. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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