In vitro Enhancement of Pancreatic β Cells MIN6 Proliferation by Insulinotropic Gymnema sylvestre Aqueous Extracts: Evidence-based Regenerative Therapeutic Capacity of a Medicinal Herb

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ABSTRACT

Aim: To show that Gymnema sylvestre (Roxb.) Asclepiadaceae not only has antidiabetic propensities, but it most likely works by regeneration of pancreatic β cells which is imperative in anti-obesity-diabetes therapeutic applications of medicinal plants.

Study Design: The present study design investigated the effects of G. sylvestre leaves crude...
aqueous extracts (AEs), traditionally utilized in diabetes treatment, on the pancreatic β-cell MIN6 proliferation and insulin secretion and extrapancreatic dietary carbohydrate and lipid digestion

**Place and Duration of Study:** Faculty of Pharmacy, The University of Jordan, 2008-2012.

**Results:** Comparable to GLP-1 (500 nM) pancreatic proliferative capacity; G. sylvestre AE concentrations (0.01 and 0.1 mg/mL) induced MIN6 monolayers expansion by respective 130.3% and 127.4% (P<0.001 vs. spontaneous control). Like L-alanine (10 mM) insulinotropic efficacy and without exerting cytotoxicity, glucose-stimulated insulin secretion was potentiated by G. sylvestre AEs (5, 10 and 25 mg/mL) (711.0%, 843.0% and 906.5%, respectively, P<0.001 vs. basal control). The potent plants’ insulin secretory bioactivities were abolished in the depleted Ca²⁺ conditions (P<0.001). Similar to orlistat antilipolytic efficacy, pancreatic lipase I₅₀ value for G. sylvestre AEs was 106.3±7.2 µg/mL. Unlike acarbose (100 µg/mL) dual inhibition of α-amylase/α-glucosidase, G. sylvestre AE was inactive at used doses. Dissimilar to guar gum (50 mg/mL) diffusional hindrance in a simple dialysis model, G. sylvestre AEs (10, 25 and 50 mg/mL) proved inactive. This *in vitro* ineffectiveness was mirrored in respective *in vivo* oral carbohydrate tolerance tests in overnight fasting normoglycemic rats.

**Conclusion:** This evaluation has revealed that G. sylvestre leaves AEs augmented β-cell expansion and potentiated glucose-evoked Ca²⁺-regulated insulin secretion; combined with impressive antilipolytic activity. These actions depend on the bioactive water soluble phyto-principles intact absorption and potentiated glucose metabolism.

**Keywords:** Gymnema sylvestre; β-cell regeneration and proliferation; pancreatic insulin secretion; α-amylase/α-glucosidase; pancreatic triacylglycerol lipase.

### 1. INTRODUCTION

Diabetes is a chronic and progressive metabolic disease characterized by impaired β-cell function, and reduced insulin sensitivity and secretion [1,2]. It is recognized as a worldwide major health problem as well as in Jordan [3]. Oral hypoglycaemic sulphonylureas have found widespread application in drug therapy of type 2 diabetes, mainly due to a direct stimulation of insulin secretion [4]. Nevertheless, chronic sulphonylureas therapy tends to progressively fail following direct desensitization and decline in beta cell K_{ATP} channel activity [5]. This further dwells upon the need for discovery of novel insulinotropic entities from medicinal plants reputed for their antidiabetic efficacies within traditional ethnomedicine practices. Literature surveys demonstrate the benefit of several ethnomedicinal principles as antidiabetic agents evaluated in the form of their crude extracts and/or isolated pure compounds with varying degrees of hypoglycemic or antihyperglycemic bioactivities [6,7]. Evidently, multiple medicinal and edible herbs, either indigenous or imported, were promoted locally for diabetes traditional medicine. These were closely linked to appreciable prevalence of herbal use among diabetes patients on conventional medicaments in Jordan [8]. Principally, *G. sylvestre* along with its phytochemistry have been long recognized for their antidiabetic pharmacological properties in multiple traditional medicine systems [9-14]. In both type 1 and type 2 diabetes, beta cell mass and function are decreased to varying degrees. Therefore, islet cell replacement or regeneration therapy may thus offer therapeutic benefit to people with diabetes [15-17]. It is well accepted that diabetes regenerative therapeutics can be obtained from plants with proliferative capacity to induce expansion and rejuvenation of pre-existing pancreatic β-cells [18-27].

Accordingly, to observe the possible changes in the proliferation, the MIN6 cells were incubated over 48 h in the presence and absence of *G. sylvestre* AEs. Furthermore, to test the possible extrapancreatic role of *G. sylvestre* AEs in viscosity-based delay and/or minimizing in postprandial hyperglycaemia and postprandial hyperinsulinemia; a simple glucose dialysis model was considered [28]. Finally, as for its physiological insulin secretagogue activities, at the submaximal stimulatory glucose concentration (5.6 mM), the anticipated potentiating of a Ca²⁺-regulated glucose-stimulated insulin secretion by *G. sylvestre* AEs was critically evaluated [29-31]. Possible *in vitro* effects of *G. sylvestre* on pancreatic α-amylase/α-glucosidase and intestinal triacylglycerol lipase were investigated [32]. Besides, complementary supportive acute *in vivo* testing was adopted. In effect, these investigations aimed to scientifically justify the
antidiabetic use of the selected medicinal herb G. sylvestre; a practice maintained in folk medicine for generations.

2. MATERIALS AND METHODS

2.1 Equipments, Chemicals and Biochemicals

Dulbecco Modified Eagle Medium (DMEM) containing 25 mM glucose was obtained from Invitrogen (USA). ELISA jumbo kit for rat high insulin was purchased from ALPCO (USA) and MTT assay kit from Promega (USA). The assays were performed according to manufacturers’ instructions. Unless stated otherwise, all reagents and chemicals of analytical grades were from Sigma (Dorset, UK). Dialysis tubing Spectra/Por® 7 Biotech Regenerated Cellulose (RC) membranes, MWCO 2000 was purchased from Spectrum Europe B.V, Breda, Netherlands. Falcon tube 50 mL was obtained from Iwaki Sctech Division, Japan. Shaking incubator was from LabTech®, Daihan LabTech Co., LTD. (Korea). d (+) glucose was procured from Riedel-deHaen, Seize (Germany). Glucose GOD-PAP (glucose oxidase - para-aminophenazone) kit was obtained from BioLabo Reagents, France. In UV determinations UV-VIS spectrophotometer (Ultraviolet – Visible) from SpectroScan 80D (UK) was used. Sonicator (Bandelin Sonorex, Bandelin electronics, Germany), rotary evaporator (Laborota 4000-efficient, Heidolph, Germany), and Accu-Chek® Active Glucose meter (Roche, Germany) were also used.

2.2 Plant Material and Preparation

Leaves crude aqueous extracts (AEs) of G. sylvestre (Roxb.) Asclepiadaceae were procured as tabulated capsules from Himalaya Drug Company, Banglore (India). Voucher specimens were deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy-The University of Jordan. For in vitro and in vivo testing, each 10 g of the capsules content were refluxed with 100 mL tap water for 15 min and kept overnight. After filtering twice, the volume of the filtered solution was increased to 100 mL with tap water to obtain 10% (equivalent to 100 mg/1mL) crude aqueous solutions [33].

2.3 Experimental Animals

The study was undertaken in the Experimental Animal Laboratory of the Faculty of Medicine, The University of Jordan. All animals were housed, fed and treated in accordance with The University of Jordan ethical guidelines for animal protection. Experimental approval was obtained from the Scientific Research Council at the Deanship of Academic Research and the Faculty of Pharmacy (registration number 218/2007-2008). Before testing, the rats were kept for one week to be acclimatized under the standard laboratory conditions. Throughout the experimentation period, healthy female Sprague/Dawley rats weighing 200-250 g were housed in single cages and were given proper pellet diet and water ad libitum. Also, rats were 14 h-fasted before in vivo blood glucose determination. Blood glucose levels from cut tail tips were determined using Accu-Chek® Active Glucose meter.

2.4 Oral Starch Tolerance Test (OSTT)

In this line of investigations; rats were divided into 5 groups (n=5-8 rats per group). At -30 min, fasting glycemia levels were evaluated and instantly acarbose (3 mg/Kg b.wt) or G. sylvestre AEs in doses 125, 250 and 500 mg/Kg b.wt were administered orally via 2 mL intra-gastric intubations under mild anesthesia. Control untreated animals were given tap water (2 mL/rat). At 0 min, all rats of five groups (plant extracts and acarbose) were given corn starch 3 g/Kg b.wt following the fasting blood glucose determination. Later evaluations of glycemia took place at 45, 90, and 135 min from 0 min [33].

2.5 Enzymatic Starch Hydrolysis In vitro

Acarbose (1000 μg/ mL) was as the reference drug [34] vs. control (tap water only) samples. A concentration range (1-50 mg/mL, n=3) of G. sylvestre AEs was assayed for comparable inhibitory efficacy of α-amylase and α-glucosidase.

2.6 Oral Glucose Tolerance Test (OGTT)

Rats were divided into 6 groups (n = 5-8 rats per group). The OGTT was carried out as the above protocol for OSTT except for glucose (3 g/Kg b.wt) utilization instead of starch and metformin (300 mg/Kg b.wt) and glipizide (600 μg/Kg b.wt) treatments as positive controls instead of acarbose [33].

2.7 Glucose Movement In vitro

Glucose solution (0.22M in 0.15 M NaCl) was added to a dialysis tubing (10 cm x 11 mm). The
tubing was sealed at both ends, and dialysed against 45 mL of 0.15 M NaCl in 50 mL tube overnight. The optimum temperature for maximum glucose diffusion was established at 37°C. Gentle shaking is used as well in a shaking incubator to simulate the effect of intestinal contractions on intestinal glucose absorption [35]. The end point of glucose diffusion equilibrium (glucose diffusion into the external solution) was found by measuring the external solution glucose content in dialysate at 0, 3, 6, 18 and 24 h time intervals. Glucose concentrations were measured in duplicates per time point sample. The assay was internally controlled using 5 mM glucose solutions prepared alongside the experimental glucose samples. Guar gum 50 mg/mL or plant AEs 10, 25 and 50 mg/mL in 0.22 M glucose in triplicates were dialysed against 0.15 M NaCl over night. The optimum temperature for glucose diffusion was found at 37°C with gentle shaking and a parallel plant-free (negative) control was included [28].

2.8 Pancreatic β-cell Viability and Proliferation Assays in vitro

Cell viability was assessed by a MTT kit. MIN6 cells were subcultured on 96-well plates (10,000 cells/well) in growth medium containing different concentrations of G. sylvestre AEs (0.01-0.1 mg/mL, n=4). After 48 h of incubation, the effects of the plant extracts on cell viability were evaluated according to kit's manufacturer instructions. Proliferation of MIN6 cells was evaluated with a colorimetric ELISA- based BrdU incorporation kit (Roche, Germany). In brief, MIN6 cells (young passage) were seeded onto 96-well plate (10,000 cell/well density) and left to adhere overnight. They were serum starved 24 h before 48 h treatments with G. sylvestre AEs concentrations (0.01-0.1 mg/mL) or GLP-1 500 nM [22] (n=3). BrdU dye 10 µM/well was added to culture medium of different treatment wells and incubated for the second 24 h of the 48 h-chronic incubation time. The rest of the assay was performed in accordance with manufacturer instructions.

2.9 MIN6 Cell Culture and Insulin Secretion Static Incubation Experiments

Pancreatic β-cells MIN6 (passage 39-45) were maintained in DMEM containing 15% Foetal Bovine Serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, 100 µg/mL L-glutamate, and 5 µL/L β-mercaptoethanol in a 37°C humidified atmosphere with 95% air and 5% CO2. The culture medium was changed every 48-72 h [36]. In testing the Glucose Stimulated Insulin Secretion (GSIS) from MIN6, cells were cultured in 96-well plates at density 50,000 cell/well until 80% confluent. On the day of experiment, growth medium was removed and the cells were washed with Phosphate-Buffered Saline (PBS). Cells were preincubated for 1 h at 37°C in 5% CO2 in HEPES-balanced Krebs-Ringer phosphate buffer (KRH) composed of (in mM) 129 NaCl, 5 NaHCO3, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 10 HEPES, 2.5 CaCl2 and 0.1% BSA (pH 7.4, NaOH) supplemented with 1.1 mM glucose. Incubation medium was removed, and the cells were washed once in glucose free KRH. Subsequent test incubations in 5.6 mM glucose - KRH alone (untreated negative control) or supplemented with appropriate treatments (L-alanine 10 mM or G. sylvestre AEs 0.01-25 mg/mL, n=4) were performed for another 1h. L-alanine was the reference robust and powerful stimulant of insulin secretion from pancreatic beta cells [28-30]. Cell viability was assessed post 1 h-acute incubations using MTT assay. When investigating the effects of extracellular Ca2+-free incubations on pancreatic GSIS, 2.5 mM CaCl2 was removed from KRH buffer preparations, so that cells were incubated in a Ca2+-free KRH in the same panel of GSIS studies described previously. For all experiments, incubation medium was collected and stored at -20°C for a subsequent ELISA determination of the amount of secreted mouse insulin.

2.10 Preparation of G. sylvestre AEs and Orlistat for In vitro Pancreatic Lipase (PL) Activity Assay and Spectrophotometric Quantification of PL Inhibition by Test Extracts

Each of the tested AEs was initially dissolved in Tris-HCl buffer (2.5 mM (Promega, USA), pH 7.4 with 2.5 mM NaCl) to give five initial stock solutions of 6.25-100 mg/mL. Subsequently, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range 125-2000 µg/mL (n=3). Extracts were prepared according to the traditional indications of use, thus dimethylsulfoxide (DMSO) or any other organic solvent; even to the minimum concentration, was avoided [37]. Finally, orlistat, the reference drug (in DMSO; 1 mg/mL), was prepared in six different stock solutions with a concentration range of 0.625 - 20 µg/ mL [38]. Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final
concentration range of 0.0125 – 0.4 µg/mL. According to Bustanji et al. [39], *In vitro* enzymatic PL activity was assayed. Subsequent determinations were undertaken for the tested extracts in comparison to control evaluations, to calculate the concentration required for PL 50% inhibition (IC$_{50}$).

### 2.11 Statistical Analysis

Results are presented as mean ± S.E.M (Standard Error of the mean) (n=3-4 determinations). Statistical differences between different treatment groups and respective controls were evaluated using ANOVA (one way analysis of variance) followed by Dunnett post test (GraphPad Prism Software 3.02, USA). Results were significantly different if $P<0.05$ and highly significantly different if $P<0.001$.

### 3. RESULTS AND DISCUSSION

#### 3.1 Lack of Extrapancreatic Modulation of Enzymatic Starch Digestion, Glucose Movement, or Oral Carbohydrate Tolerance Tests by *G. sylvestre* AEs

New approaches to the prevention/modulation of postprandial hyperglycemia may emerge from the therapeutic use of α-amylase and α-glucosidase inhibitors [33,34]. Hence, the present *in vivo* and *in vitro* experiments evaluate the acute (up to 165 min carbohydrate tolerance testing in normal animal models) postprandial antihyperglycemic activity of the *G. sylvestre* with claimed antidiabetic activity exploring the enzymatic starch digestion and glucose diffusion modalities as possible antidiabetes action modes. Fig. 1a demonstrates that at -30 min time point acarbose 3 mg/Kg b.wt administration reduced highly significantly the starch induced hyperglycemia at 45, 90, and 135 min, thus evoking highly substantial reduction ($P<0.001$) of the overall glycemic excursion compared to controls. *G. sylvestre* treatment groups, nevertheless, did not minimize effectively the overall glycemic excursion versus the control and drug treated animals (the same figure). Fig. 1b confirms the *in vitro* lack of the effect of *G. sylvestre* AEs on enzymatic starch digestion. Fig. 2a displays that 30 min pre-glucose-load treatments with metformin (300 mg/Kg b.wt) and glipizide (0.6 mg/Kg b.wt) minimized highly markedly ($P<0.001$) the overall glycemic excursions in OGTTs compared to control normal rats. It further highlights the substantial ($P<0.001$) antihyperglycemic efficacies of both oral antidiabetic therapeutics at 45, 90, and 135 min following sugar load. Oral administration of *G. sylvestre* AEs had no marked improvement of glucose tolerance AUCs, unlike the therapeutic efficacies of metformin or glipizide vs. control glucose-only fed rats. Fig. 2b illustrates that using a simple diffusion model, mean AUC, for the viscous water-soluble gel forming guar gum (50 mg/mL) was decreased highly distinctly by 30.9±2.5% ($P<0.001$, n=3) compared to overnight negative control. Guar is a natural oral antidiabetic and classical positive control [22]. *G. sylvestre* AEs lacked any statistically substantial glucose diffusional hindrances into external solution across dialysis membrane (with respective 5.9±0.5% and 1.9±0.34% AUC reductions, $P>0.05$ vs. controls’ AUCs, the same figure).

#### 3.2 Pancreatic β-cell Monolayers’ Expansion Modulated by *G. sylvestre* AEs

Approximately 80% of the investigated traditional plants used for the treatment of diabetes demonstrated some antidiabetic activity; thus plants can represent a vast pool of potentially useful dietary supplements for improving blood glucose control and preventing diabetes long-term complications [21-23,29-30,33,34,40]. Compared to control untreated cells, the MTT method revealed that 48h post seeding *G. sylvestre* AEs at doses 0.01-0.1 mg/ml preserved cell integrity (Table 1). A colorimetric immunoassay of BrdU-incorporation into MIN6 β-cell genome was recruited to ascertain proliferative principles of chronic plants treatments. Fig. 3 demonstrates that GLP-1 (500 nM) highly significantly promoted a maximal extent of BrdU incorporation into MIN6 β-cell genome which is 1.3 folds equally ($P<0.01$ vs. basal controls, the same figure).

Obviously, *G. sylvestre* not only was ascribed antidiabetic property, but also it works most likely by regeneration of the pancreatic β-cells which is very imperative in light of damaged pancreatic islets in diabetes patient. In both type 1 and type 2 diabetes; insufficient numbers of insulin-producing beta cells are a major cause of
defective control of blood glucose and its complications [41,42]. Accordingly, therapies that increase functional beta-cell mass may offer a cure for diabetes. Efforts to achieve this goal explore several directions. Based on the realization that beta cells are capable of significant proliferation throughout adult life, the enhanced proliferation of beta cells in vivo or in vitro is pursued as a strategy for regenerative medicine for diabetes [43]. This study clearly links our substantially outstanding in vitro outcomes with the extensively reported in vivo regenerative outcomes for G. Sylvestre [9-12, 44-46] in diabetes animal models and patients. Thus it provides potentially promising avenue in the design and implications of regenerative therapies aimed at increasing beta-cell replication and mass in patients with diabetes [47]. Based, G. sylvestre is an interesting source for antioxidant phenolic phytochemicals that can be useful for various therapeutic applications [48]. Therefore it effectually could protect islets of Langerhans against diabetogenic alloxan- and STZ- induced oxidative insult [49,50] in animal models of the chronic disease course. This may have taken place via enhancing natural cellular antioxidant system [51].

Table 1. Lack of modulatory effects of G. sylvestre AEs (0.01, 0.05 and 0.1 mg/mL) on the viability of pancreatic beta-cells MIN6 in 48 h post seeding as measured by MTT kit

| Treatment           | MIN6 viability (as % control) |
|---------------------|------------------------------|
| Control incubations (plant free) | 99.5±10.4                  |
| G. sylvestre AE (mg/mL) |                             |
| 0.01                | 99.0±8.5                     |
| 0.05                | 115.4±7.2                    |
| 0.1                 | 96.0±25.9                    |

Each result (as % Control) indicates the mean ± S.E.M of four independent experiments.

Fig. 1a. Lack of modulatory postprandial antihyperglycemic effects of G. sylvestre (AEs) concentrations in mg/Kg b.wt on oral starch tolerance over 165 min and AUC in normoglycemic overnight fasting rats

*P<0.05 and ***P<0.001 compared to control untreated animals, as determined by unpaired sample t-test
Fig. 1b. Lack of in vitro inhibitory effects of *G. sylvestre* (AEs) (mg/mL) on starch digestion enzymes α-amylase and α-glucosidase

Results are mean ± SEM (n = 3 independent replicates). ***P<0.001 compared to control (drug-free or plant-free) incubations, as determined by unpaired sample t-test
3.3 Glucose-Dependent Modulation of Insulin Secretion in Pancreatic β-cell by *G. sylvestre* AE is Mediated by Ca^{2+} Dependency

A key feature of type 2 DM is that glucose fails to stimulate an adequate release of insulin from pancreatic β-cells. The stimulatory effect is mediated via the pancreatic beta cell K_{ATP} channel [52]. Binding of sulphonylureas leads to K_{ATP} channel closure, evoking membrane depolarization and subsequent opening of VOCCs (voltage operated Ca^{2+}-channels), followed by elevation of [Ca^{2+}]_{i} (intracellular Ca^{2+} concentrations) due to increased Ca^{2+} influx, ultimately leading to exocytosis of insulin from secretory granules [52].

Basic, to evaluate the insulinotropic activity of plant extracts, submaximal stimulatory glucose concentration (5.6 mM) was used in the acute culture incubations. L-alanine 10 mM was used as a positive control and it enhanced substantially (p<0.05) GSIS in MIN6 by 307.3±34.6% (n=4) following 1 h-incubations, compared to untreated (5.6 mM glucose only) controls (Fig. 4). Exceedingly superior to L-alanine, *G. sylvestre* AE concentrations 5-25 mg/mL potentiated GSIS in pancreatic MIN6 substantially by respective 711.1±39.4%, 848.0±13.8% and 906.5±44.3% (p<0.001 vs. basal control wells, Fig. 4). Significant increase of the MIN6 insulin release at lower concentrations was not detected. Cell viability, as checked by MTT assay, was unchanged over 1 h-incubations in effective concentrations' wells. Moreover the marked insulintropic trend of L-alanine was highly significantly abrogated in Ca^{2+} depleted KRH (195.7±31.6%, p<0.001), as compared to corresponding Ca^{2+} free glucose only (negative control) wells. Comparably, *G. sylvestre* (5-25 mg/mL) insulinogenic efficacies were markedly ablated (p<0.001) following Ca^{2+} absence from acute plant incubations to 170.7±7.0%, 176.5±6.7% and 183.5±7.2%, respectively, compared to respective Ca^{2+}...
buffered conditions (Fig. 4). Explicitly, G. sylvestre extracts along with phyto-constituents are appraised for their pronounced insulintropic and insulinomimetic propensities in vitro and in vivo [53-58]. As indicated lately, combination therapy markedly improves glycaemic control, thus allowing for regimens design to specifically address the diabetes underlying abnormalities [1,2]. The substantial insulinogenic properties further complement the pancreatic proliferative efficacies illustrated of G. sylvestre, thereby collectively, advocating the plant as a potential source with functional properties for active leads into anti-diabetes pharmacology and/or its adjunctive therapeutic strategies.

3.4 In vitro Inhibition of PL by G. sylvestre AEs

Exceptionally, antiobesity qualities were attributed to G. sylvestre and its phytoprinciples in animal models [59-61]. Results of G. sylvestre AEs pancreatic triacylglycerol antilipase activity in vitro are shown in Fig. 5. Orlistat's PL-IC50 value of 114.0±4.0 ng/mL, equivalent to 0.2±0.0 μM, is comparable to reported PL-IC50 values elsewhere [38].

![Graph showing the lack of in vitro effects of G. sylvestre (AEs) on the incremental AUC of 24h glucose movement](image1)

**Fig. 2b. Lack of in vitro effects of G. sylvestre (AEs) (mg/mL) on the incremental AUC of 24h glucose movement**

Results are mean ± SEM (n = 3 independent replicates). ***P<0.001 compared to control (basal) incubations, as determined by unpaired sample t-test

![Graph showing modulatory effects of G. sylvestre AE on proliferation of pancreatic β-cells MIN6 in 48 h culture incubations](image2)

**Fig. 3. Modulatory effects of G. sylvestre AE (0.01 – 0.1 mg/mL) on proliferation of pancreatic β-cells MIN6 in 48 h culture incubations as measured by a colorimetric ELISA-based BrdU incorporation kit**

Each bar indicates the mean ± S.E.M. of four independent determinations; *P<0.05 and ***P<0.001 compared to untreated (spontaneous) control conditions, as determined by unpaired sample t-test
Fig. 4. Modulatory effects of *G. sylvestre* AEs (0.01 – 25 mg/mL) on secretory function of pancreatic MIN6 β-cells. Such augmentation of GSIS (glucose stimulated insulin secretion) following acute 1h-treatments was evaluated by rat insulin ELISA. *G. sylvestre* treatment wells were co-incubated in corresponding 5.6 mM glucose. Each bar indicates the mean ± S.E.M. of four determinations. ***P<0.001 compared to respective 5.6 mM glucose (basal) control wells as determined by unpaired t-test; ΔΔΔP<0.01 and ΔΔΔΔP<0.001 compared to respective treatment conditions in the presence of 2.5 mM Ca2+.
Fig. 5. *In vitro* inhibitory effects of *G. sylvestre* (AEs) as well as orlistat concentrations in μg/mL on Pancreatic Triacylglycerol Lipase Activity

Results are mean ± SEM (n = 3 independent replicates).

Comparable to orlistat performance, a marked concentration dependent PL inhibition trend was obtained per tested extracts (the same figure). *G. sylvestre* PL-IC₅₀ value for a minimum of triplicate determinations was 106.3±7.2 μg/mL. Thus *G. sylvestre* maybe be advocated as a slimming agent and, hence, a potential candidate for obesity-diabetes prevention and phytotherapy.

4. CONCLUSION

Unprecedentedly, inhibitors of α-amylase and/or α-glucosidase should be considered whenever postprandial hyperglycemia is the dominant metabolic abnormality. Our data indicate that *G. sylvestre* AEs could not improve glucose homeostasis via delaying carbohydrate digestion or absorption significantly but it was markedly proven as antilipolytic agent. Moreover, *G. sylvestre*, via induction of β-cell mass expansion, may ameliorate pancreatic dysfunction. Distinctly, *G. sylvestre* high concentrations potentiated Ca²⁺-regulated glucose-evoked acute insulin secretion. However, further chronic subclinical testing is required to validate its clinical implementation as therapeutic agent for improvements in impaired peripheral carbohydrate tolerance and obesity-diabetes.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors declare that there are no conflicts of interests.
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