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Research Article

Polyphenols rich *Apium graveolens* leaves modulate diabetic signalling pathways PTP1B, DPPIV and GLP-1, their inhibitory effect on α-glucosidase, pancreatic lipase and α-amylase enzymes and improve insulin secretion in glucotoxicity

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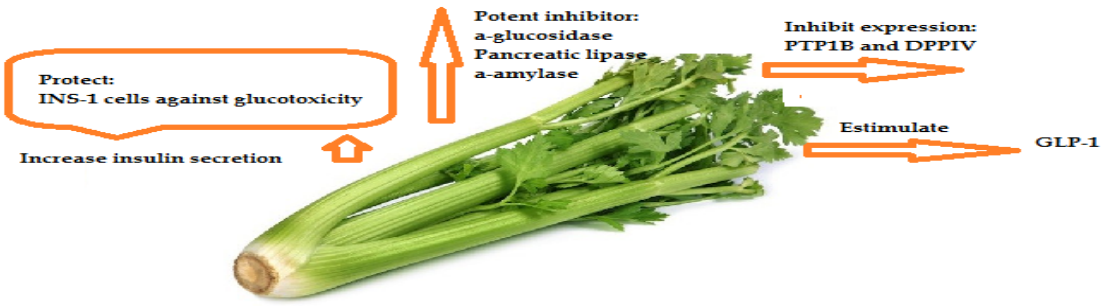
ABSTRACT

Background: The leaves of *Apium graveolens* are commercially important aliment belonging to the family Apiaceae which also are used as an ethnomedicine in the treatment of diabetes mellitus and obesity in Mexico.
Objetive: The purpuse of this study was to investigate whether the methanol extract of leaves of *Apium graveolens* (CM) can modulate diabetic signalling patways, can inhibit enzimes linked to obesity and can protectet pancreatic cells. Methods: The leaves of celery was extracted with metanol (CM). Polyphenols contents in CM were investigated by liquid chromatography-electrospray ionization mass. The ability of the compounds to inhibit formation of expression of protein tyrosine phosphatase (PTP1B) and dipeptidyl peptidase IV (DPPIV) enzyme were evaluated in vitro models. The effect of CM on glucagon-like-peptide-1 **(**GLP-1) secretion in GLUTag cells and INS-1 cell against high glucose-induced glucotoxicity were measured.
Results: Apigenin, luteolin, kaempferol, apiin, rutin, caffeic acid, ferulic acid, chlorogenic acid, coumaroylquinic acid, and p-coumaric acid as the major polyphenols contained in CM. In vitro assays CM was more potent inhibitor of α-glucosidase than pancreatic lipase and α-amylase enzymes. Furthermore, CM exhibited highest inhibitory activity against expression of protein tyrosine phosphatase (PTP1B) and dipeptidyl peptidase IV (DPPIV) enzyme. In addition, CM stimulate glucagon-like-peptide-1 **(**GLP-1) secretion in GLUTag cells and protects INS-1 cell against high glucose-induced glucotoxicity increasing insulin secretion.
Conclusions: These finding indicate that CM possess anti-diabetic effects since can act on different target affected in this disease with few side effects. These results show that *A. graveolens* is a potential functional food for treatment of type 2 diabetes.

Keyword: Apium graveolens, polyphenols, anti-diabetic

Graphical Abstract



1. INTRODUCTION

Hyperlipidemia and hyperglycemia are major risk factors for developing of cardiovascular diseases and diabetes mellitus [1]. One of the strategies to reduce obesity is decrease dietary fat, carbohydrate and it absorption in the small intestine [2] which causes an attenuation in hypercholesterolemia, hypertriacylglycerolemia, formation of reactive oxygen species and postprandial hyperglycemia, hence reduced risks of the development of diabetes and cardiovascular diseases [3]. Based on this principle find the well-known pancreatic lipase inhibitor (orlistat) and α-glucosidase inhibitor (acarbose) are clinically used for treatment of hyperlipidemia and hyperglycemia. However, α-amylase, a salivary enzyme also participate in the breakdown of carbohydrates within simple molecules [4].

PTPs are enzymes that catalyze protein tyrosine dephosphorylation. There are more than one hundred types in humans functioning as either positive or negative modulators in several signal transduction pathways [5]. Protein tyrosine phosphatase (PTP1B) belonging to the family of PTP is a negative regulator of insulin receptor (IR) signaling. PTP1B is considered a drug target for the treatment of obesity and type 2 diabetes [6].

Dipeptidyl-peptidase IV (DPPIV) inhibitors have been used for treatment of type 2 diabetes, known as gliptins which prolong post-prandial insulin action and increasing incretin levels related to metabolic syndromes and obesity [7].

Glucagon-like peptide-1 (GLP-1) is a potent gluco-incretin hormone, plays a important role in insulin secreting effect, pancreatic beta cell proliferation, and survival [8]. Recently, GLP-1 based therapies have been used for the treatment of diabetes, showing clinically modest effects [9].

The increase adipose tissue mass in obesity lead to increased secretion of proteins that deregulates insulin sensitivity, glucose homeostasis, cytokines and adipokines affecting pancreatic β-cells performance [10]. Therefore, relevant studies from plant-derived polyphenols have shown to protect islets against streptozotocin and cytokines. Among them polyphenols from *Broussonetia papyrifer* exert anti-diabetic activity through the inhibition of α-glucosidase and PTP1B [11].

Apium graveolens L. (Celery) is a biennial plant and a commercially important spice belonging to the family Apiaceae. Celery is used as fresh plant, seeds, stalk, oleoresin and oil for flavoring of foods. Celery extracts are reported to possess many pharmacological properties, such as attenuates liver enzymes, and improves lipid profile in cholesterol-fed diets [12] In another study, the methanolic extract of *A. graveolens* seed have protective effect on liver damage [13]. Also, inhibiting oxidant process due to the constituents derivatives of methoxy-phenyl chromenone and L-tryptophan [14]. Nonpolar extract of root and bulbs of *A. graveolens* showed significant cytotoxicity effect [15]. Apiuman, a pectic polysaccharide isolated from *A. graveolens* increased interleukin-10 production and decrease the interleukin-1β and diminish the neutrophils migration, causing anti-inflammatory activity [16]. The antidiabetic, lipid profile and antiglycation effect of the chloroform extract of the celery leaves was determined in previous studies performed by us [17]. In another study, was determined that the hydroalcoholic extract of celery decreased low-density lipoprotein, triglycerides and colesterol [18]. The mechanism of this hypocholesterolemic effect is due to content of compounds with sugar/amino acid moiety in the extract causing an effect on bile acid secretion [19]. In the present study, antidiabetic effect of *Apium graveolens* in vitro are discussed.

2. METHODS

2.1. Chemicals and reagents

p-nitrophenyl glycoside, 3,5-dinitro salicylic acid, p-nitrophenyl laurate, p-nitrophenyl phosphate, , 3-(N-Morpholino) propanesulfonic acid, 3-hexadecanoyl-5-hydroxymethyl tetronic acid (RK-682), methylglyoxal (MG), fetal bovine serum (FBS), trypsin, crystal violet, sodium dodecylsulfate, apigenin, luteolin, kaempferol,

apiin, rutin, caffeic acid, ferulic acid, p-coumaric acid, DPPIV enzyme, Glyo-Pro7-amido-4-methylcoumarin hydrobromide, Lithocholic acid, were products of Sigma Chemical Co. (St. Louis, MO). Pancreatic lipase, α-amylase, α-glucosidase, PTP1B (human, recombinant) were purchased from BIOMOL International LP (USA), GLUTag and INS-1 cells were acquired in Invitrogen (Carlsbad, CA, USA), RPMI-1640 (Gibco BRL, NY, USA), DMEM (Gibco BRL, NY, USA), GLP-1 Total ELISA kit, Millipore (Merck, USA), dipeptidyl peptidase-4 inhibitor and glucagon-like peptide-1 (7–36) amide and antibody against actin ELISA kit (Alpco Diagnostics, NH, USA). BCA protein assay (Pierce, Rockford, IL, USA), Rat/mouse insulin was measured by ELISA (Millipore, Billerica, MA, U.S.A). RPMI medium, streptomycin and penicillin were purchased from HyClone Laboratories (Lo-gan, UT, USA). P32/98 {(3N-[(2 S, 3 S)−2-amino-3methyl-pentanoyl]−1,3-thiazolidine) hemifumarate was purchased from Tocris Bioscence (MN, USA). All other chemicals and reagents used were of reagent grade.

2.2. Plant material

Fresh celery was collected in Amecameca, Mexico State. Voucher specimen (6543) is kept in the Herbarium of Escuela Nacional de Ciencias Biologicas-IPN. The fresh materials for the celery, (stalk, and leaves) were cut into small pieces and dried at 50 ºC, for 4 h and then plant materials were finely powdered and passed through a mesh #40-60 sieve prior to extraction [20].

2.3.Plant extraction

1 kg of the sample's powder was transferred into a 10-L reaction vessel, and then 4 l of methanol was added. The mixture was refluxed for 3 h and filtered using Whatman filter paper. The extraction was repeated twice. The filtrates were combined and concentrated to dryness using a rotary evaporator at 40-50°C. The crude extract (CM) was weighed, kept in tubes wrapped with aluminium foil to reduce the risk of oxidation and stored in deep freeze.

2.3. Characterization of polyphenols by liquid chromatography-electrospray ionization mass (LC/ESI-MS)

The methanol extract was subjected to Ultimate 3000 C18 semi prepared column (10 mm x 150 mm; DIONEX, USA), and eluted with a gradient mobile phase of methanol:ethyl acetate(1: 9) → methanol:ethyl acetate (1:1) → methanol at a flow rate of 30 ml h^{-1}, in the period of 60 minutes, being monitored at 215 nm. Fractions were checked by silica gel thin-layer chramatography (TLC) and eluted with ethyl acetate : methanol (9 : 1) after the TLC was visualised at UV 365 nm and spraying with a solution of $AlCl_3$ in ethanol. LC/ESI-MS (Agilent 1100 HPLC) was used for phenolic compounds identification using a reversed-phase BDS Hypersil C18 (3 urn particle size; 150 x 2.1 mm, i.d.) column. The solvent system was a mixture of water:acetonitrile (A) and formic acid (B), with the following gradient of A: 0 min, 20%; 10 min, 25%; 15 min, 100%. Elution was carry out with solvent flow rate of 0.3 ml min^{-1}. Mass spectra were acquired using electrospray ionization in the positive (PI) mode, at low (70 V) and high fragmentation voltages (250 V) for ionization mode. The mass spectra were recorded for the range of *m/z* 100-800.

2.4. Determination of α-glucosidase inhibitory activity

The method described by Tsujii et al [21]. was used to evaluated α-glucosidase inhibitory activity of the extract. Briefly, α-glucosidase (0.2 U/mL) was dissolved in a phosphate buffer (50 mM, pH 6.7) and pre-heated at 37 ºC for 10 min. Specific concentrations of the extracts were then added (1.25. 2.5, 5.0, and 10.0 mg/mL). To initiate the enzyme reaction, 1 mL of 1 mM p-nitrophenyl glycoside, was added to the reaction system and incubated in a water bath for 20 min at 37 ºC. Then, reaction was terminated for the addition of 1 mL anhydrous methanol. Enzymatic activity was quantified by measuring the absorption at 405 nM.

2.5. Inhibition of α-amylase

Starch solution (0.5% w/v) was prepared by stirring 0.125 g of potato starch in 25 mL 20 mM sodium phosphate buffer with 6.7 mM sodium

chloride, pH 6.9 in boiling water bath for 15 min. The enzyme solution was prepared by dissolved 0.5 mg/mL of α-amylase in sodium phosphate buffer (pH 6.9) this solution was pre-incubated at 25 °C for 10 min. Starch solution (1 mL) was mixed with increasing concentration at 50, 100, 200, 400, and 800 µg/mL of the inhibitor (0.1 mL) and to this 1 mL of enzyme solution was added and leave to react at 25° C for 10 minutes with starch solution. The reaction was terminated by adding 500 µL of 3,5-dinitro-salicylic acid (DNS) reagent after incubation. The tubes were then incubated for 5 min in boiling water and cooled to room temperature. To the reaction mixture was added 5 mL of distilled water and the absorbance was measured in a spectrophotometer at 540 nm using [22]. Acarbose (0.15 mg/mL) was used as a positive control.

2.6. Determination of pancreatic lipase inhibition activity

According to the method of McDougall [23] was evaluated pancreatic lipase inhibition activity. Pancreatic lipase were prepared using 0.1 M Tris buffer at pH 6.9. The p-nitrophenyl laurate (pNP; 0.08%, w/v) was dissolved in 5 mM sodium acetate (pH 5.0) containing 1% Triton X-100. The mixture contained 400 µL assay buffer, 50, 100, 200, 400, and 800 µL of CM (0.1 mg/mL) and 150 µL of 10 mg/mL lipase then was heated for 10 min at 37 °C. Then, 450 µL of pNP laurate was added and incubated for 2 h at 37 °C in darkness. The samples were centrifuged for 3 min at 16,000 rpm and the absorbance was measured in a spectrophotometer at 405 nm. Orlistat (0.065 mg/mL) was used as a positive control.

2.7. PTP1B Inhibition Assay

PTP1B enzyme activity was determined using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate [24]. To each 96-well (200 µl) were added 2 mM p-NPP and PTP1B (0.05–0.1 µg) in a bu□er containing 0.1 M NaCl, 50 mM citrate (pH 6.0), 1 mM dithiothreitol (DTT) and 1 mM EDTA with or without test extract. Further incubation for 30 min at 37 °C, the reaction was terminated with 10 M NaOH. The amount of produced *p*-NPP was measured by the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM *p*-NPP was corrected by determining the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. 3-hexadecanoyl-5-hydroxymethyl tetronic acid (RK-682) and ursolic acid known phosphatase inhibitor were used as positive control.

2. 8. DPPIV enzyme assay

Dipeptidyl peptidase IV (DPPIV) enzyme at 0.1 mg/ml was incubated with CM at different concentrations (50, 100, 200, and 300 µg/mL) at 37 °C for 30 min in a humidified atmosphere containing 5% CO2. Then was added glyo-Pro7-amido-4-methylcoumarin hydrobromide (10 µM, 25 mg) and incubated at 37 °C for 30 min, P32/98 {(3N-[(2 S, 3 S)−2-amino-3methyl-pentanoyl]−1,3-thiazolidine) hemifumarate, (20 µg) was used as a standard control. The hydrolysis of substrate by DPPIV enzyme was determined at 355 nm of extinction and 460 nm of emission [25].

2.8. Cell culture

GLUTag and INS-1 cells were cultured in DMEM high glucose with hygromycin 600 µg/ml, Zeocin™ 100 µg/ml, HEPES (pH 7.3) 25 mM, non-essential amino acids 0.1 mM, 100 µg/ml streptomycin, 100 U/ml penicillin, and 10% fetal bovine serum. INS-1 rat insulinoma cells were maintained in RPMI-1640 supplemented with 1 mM sodium pyruvate, 50 µM beta-mercaptoethanol, 100 µg/mL streptomycin, 100 U/mL penicillin, 10% fetal bovine serum, and 11 mM glucose. GLUTag mouse enteroendocrine L cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with100 µg/mL streptomycin, 100 U/mL penicillin, 10% fetal bovine serum, and 5.5 mM glucose.

2.9. Measurement of glucagon-like peptide-1 (GLP-1) secretion GLUTag cells

GLUTag cells were seeded (1×10^5 cells/well) whitin 24-well culture plates and incubated for 24 h. The cells were washed with KRB buffer (0.2% BSA, 20 mM HEPES, 25.5 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.16 mM $MgSO_4$•7(H_2O), 1.2 mM KH_2PO_4, 4.7 mM KCl, and 0.114 M NaCl, pH 7.2) and then incubated in KRB buffer containing 0.2 µM dipeptidyl peptidase-4 inhibitor, 10 mM glucose and MC for 30 min. The supernatants

were collected and the total GLP-1 secretion was measured using an GLP-1 Total ELISA KIT, according to the manufacturer's instructions. Lithocholic acid (LCA; 30 μM) was used as a positive control.

2.10. Glucose induced insulin secretion

INS-1 cells (3x105 cells/well) were seeded in 24-well plates and grown for 72 hours. The day of the experiment, cells were washed with glucose-free Krebs solution and pre-incubated for 2 h with 5% CO2 at 37 ˚C in Krebs buffer and MC at 50 μg/mL. Insulin secretion was determined in the presence of 5.5, 11, and 16.5 mM/L glucose in the presence or absence of GLP-1 (10 nmol/L). After 60 minutes at 37˚C, aliquots of supernatant were taken for measuring insulin concentration, while total protein content was determined by using BCA protein assay and Rat/mouse insulin was measured by ELISA Kit according to the manufacturer's instruction.

2.11. Statistical analyses

Data are presented as mean ± SE. The significance of differences was analyzed by one-way ANOVA with the post-hoc Duncan procedure using SPSS ver. 10.0 (SPSS Inc.) The value of statistical significance was set at $p<0.05$.

3. RESULTS

Data from the LC-DAD-ESI/MS were used to identify the five phenolic compounds and five flavonoids as most abundant contained in CM. A comparison of the UV, Rt, and MS data with those of known standards established that apigenin, luteolin, kaempferol, apiin, rutin, caffeic acid, ferulic acid, chlorogenic acid, coumaroylquinic acid, and p-coumaric acid are the major phenolic acids and flavonoids are contained in celery [26]. After verification was carried out by spiking of each of the standards with the extract. The identification was verified in each case. Date obtained are similar to those compounds from celery [27].

Fig 1A demonstrates that CM had significantly (p <0.05) α-glucosidase inhibitory activity showing like α-glucosidase inhibitory activity than acarbose. The results in Fig 1B demonstrate that the percentage inhibition of CM against α-amylase was significantly lower than acarbose. The results of pancreatic lipase inhibition at different concentrations of CM are summarized in Fig 1C where showed a strong inhibitory potential against pancreatic lipase with ranging from 21.17 to 68.19 %. However, CM was less potent on inhibition of pancreatic lipase to the concentrations tested than that of orlistat (73.25%).

This study has shown that a mixture of flavonoids and phenolic compounds (CM) inhibited the enzymatic activity of PTP1B in vitro. The half maximal inhibitory concentration IC_{50} value of CM was determined. CM exhibited the highest inhibitory effect against PTP1B with a IC_{50} value of 13.12 μg/mL in contrast with RK682 with a IC_{50} value of 2.17 μg/mL and ursolic acid with a IC_{50} value of 9.11 μg/mL used as standard controls.

CM from *A. graveolens* significantly (p<0.05) inhibited DPPIV enzyme (Fig. 2). Date shows that the e□ect of di□erent concentrations of CM produced in a dose-dependent manner inhibition of DPPIV in comparison to the DMSO control and the commercially inhibitor (P32/98). Extract significantly inhibited PTP1B enzyme by 62.60% at 300 μg/mL compared to P32/98 (73.5%).

The stimulatory effects of CM on substantial secretion of GLP-1, we assayed in GLUTag cells. CM at concentrations of 20, 30, 50 and 75 μg/mL stimulate GLP-1 secretion substantially in murine GLUTag cell line. A quantity of 75 μg/mL of CM produced a significant stimulation of GLP-1 of 71.21% (Fig 3).

Insulin secretion was measured from INS-1 cells cultured in response to 5.5, 11, and 16.5 mM/L of glucose for five days (Fig. 4). Insulin secretion in response to 5.5, 11 and 16.5 mM/L glucose significantly decreased when compared with the cells control. When CM was included in the culture of INS-1 cells, insulin secretion in response to 5.5, 11 and 16.5 glucose significantly increased as compared with the cells cultured in the absence of CM (Fig. 1). These findings demonstrated that CM protects INS-1 cell against high glucose-induced glucotoxicity. CM is a potent agonist of GLP-1, which increased in a dose-dependent manner insulin secretion and

enhanced glucose-stimulated insulin secretion in INS-1 cells in response to low or moderately high concentrations of glucose.

4. DISCUSSION

One proposal for treatment of the diabetes mellitus is through inhibition of key carbohydrates digesting enzymes, α-glucosidase and α-amylase to retard the absorption of glucose. The present study showed that methanol extract from *A. graveolens* leaves moderately inhibit α-amylase activity while the methanol extract CM strongly inhibits α-glucosidase activity. According to previous studies the best clinical results were found when α-glucosidase and α-amylase inhibitors have a strong inhibitory activity against α-glucosidase and a mild inhibitory activity against the α-amylase [28]. Celery had demonstrated these effects and hence could be used to enhance postprandial hyperglycemia in diabetic patients.

Qsymia and orlistat are drugs on the market used to fight obesity which have several side effects. Thus vegetables and fruits with medicinal effects do not have side effects being compatible with our organism are preferred for long term consumption. CM exhibited the highest inhibitory activity against lipase, to inactivate digestive lipase can decrease incidence of diseases caused by diets rich in fats decreasing obesity.

PTP1B plays a important role in metabolism, equilibrating leptin in obesity and equilibrating insulin in diabetes, thus is an important target for the treatment in both obesity and type 2 diabetes. Some PTP1B inhibitors are in study in clinical research as trodusquemine, developed by Genaera Corporation. The current therapeutic class of the metabolic syndrome includes pramlintide, thiazolidinedione, GLP-1 agonist, insulin, glinide, sulfonylurea, metformin, α-glucosidase inhibitor etc [29] which have a wide variety of side effect such as hypoglycemia, aterosclerosis, insulin resistance, increased risk of congestive heart failure, edema, obesity, abdominal pain, nausea, diarrea, increase in the frequency of bowel movement and weight gain [30] leading to inadequate treatment to control glucose levels over a long period of time. Therefore, new treatments are emerging to improve diabetes therapy. Among these, DPP-IV inhibitors are widely explored and most safe. DPP-IV inhibitors inhibit α-cell secretion, improve β-cell function increased its mass and improving insulin sensitivity. Therefore, DPP-IV inhibitors are orally active, safe, tolerable, efficient and can improve considerably treatment of type 2 diabetes. Due to the lack of effective medications for the treatment of diabetes, the target of modern antidiabetic therapy is inhibition of DPPIV.

GLP-1 is secreted from enteroendocrine L-cells, stimulating proliferation of pancreatic β-cells and glucose-dependent insulin secretion [31]. Increased GLP-1 secretion induced by CM may improve level and plasma insulin and plasma glucose. It is well known that GLP-l display potent glucose-lowering and insulinotropic effects through so-called incretin effect ameliorating glucose-stimulated insulin secretion [32]. In addition, chronic GLP-l increase may reduce food intake, inhibit glucagon secretion, slow gastric emptying, produce insulin-like effects increasing insulin sensitivity and enhance β-cell function [33]. In addition, GLP-l increases glucose transport in skeletal muscles and liver, as well as increases glycogen synthesis [34]. During the progression of type 2 diabetes, pancreatic β-cell function and mass decrease considerably and this leads to a growth in the search for some therapeutic agents can protect β-cell function [35]. GLP-1 is an incretin hormone secreted in response to ingesting nutrients. When GLP-1 binds to its cognate receptor, insulin secretion is stimulated by glucose improving β-cell function. Furthermore, GLP-1 enhance the survival of β-cells and avoid its apoptosis [36]. These findings demonstrated that *A. graveolens* act as a GLP-1 secretagogue, which consumed in the daily diet can help to prevent diabetes.

In a large dense core vesicles in the pancreatic β-cells the insulin is stored by exocytosis is secreted in response to different hormonal modulators and nutrient stimuli [37]. In this exocytosis process, which is a key step in achieving a high level of insulin in the blood after food intake to maintain

glucose homeostasis, the secretory vesicles to release insulin extracellular space fuse with the cell membrane [38]. In addition, in homeostatic condition the 80% of total glucose removal is performed by skeletal muscle, and the rest by other insulin sensitive tissues and adipose cells [39]. In this study established in INS-1cell line whether CM protected pancreatic β-cells against glucotoxicity. Treatment with 25 and 50 µg/mL CM increased cell viability and insuline level in INS-1 cells pretreated with high concentrations of glucose. Finding suggested that CM can facilitate the insulin containing vesicle exocytosis and improved insulin secretion.

5. CONCLUSION

Chemical study on the leaves of CM from *Apium graveolens* resulted in the identification of five phenolic acids and five flavonoids as main components which may be the compounds responsible for antidiabetic effect of *A. graveolens*. CM displayed significant antidiabetic properties involving pancreatic lipase, α-amylase, α-glucosidase, PTP1B, DPPIV inhibition, stimulate secretion of GLP-1, and protected INS-1 cell against high glucose-induced glucotoxicity. The diet is an important part in the antidiabetic treatment accordingly dietary intervention could reduce diabetic complications which is a powerful help to conventional antidiabetic treatment. In daily food preparation *A. graveolens* is used in many countries. Thus, this is a natural food that can be administered to treat and prevent type 2 diabetes.

Conflict of Interest: The authors declare that they have no conflict of interest

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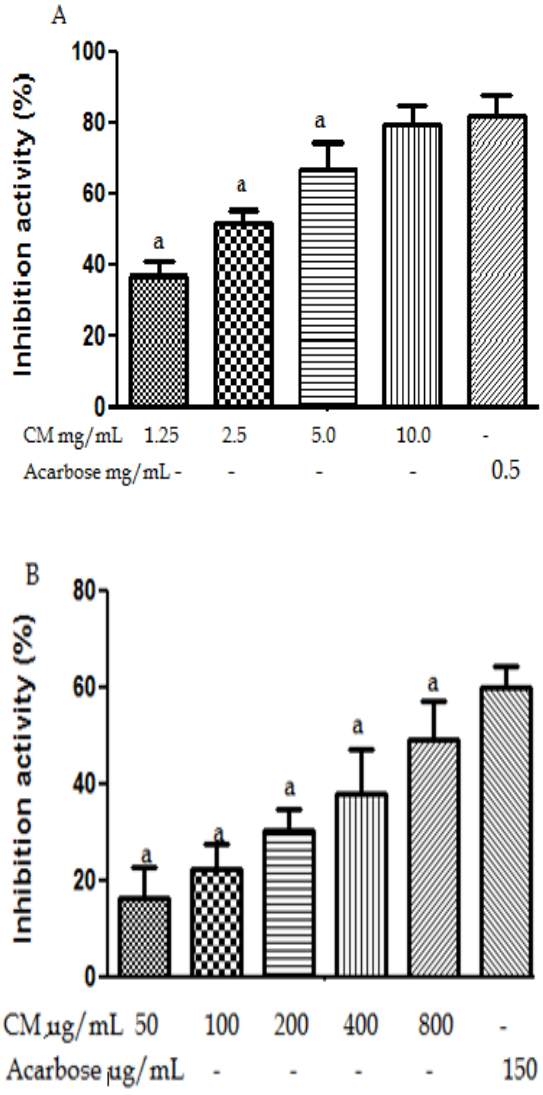

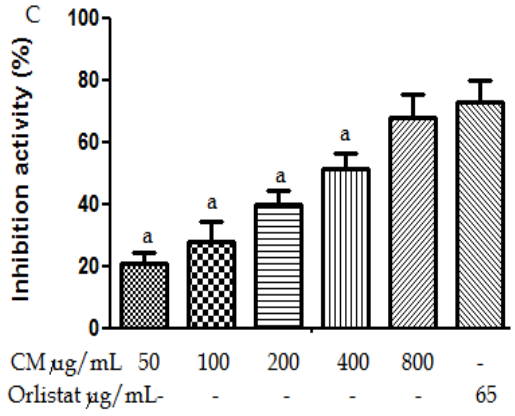

Fig 1. Percent inhibition of CM against α-glucosidase (A), α-amylase (B) and pancreatic lipase (C). Results are represented as mean ± SD (n=4). [a]p value<0.05 versus standard acarbose and orlistat respectively.

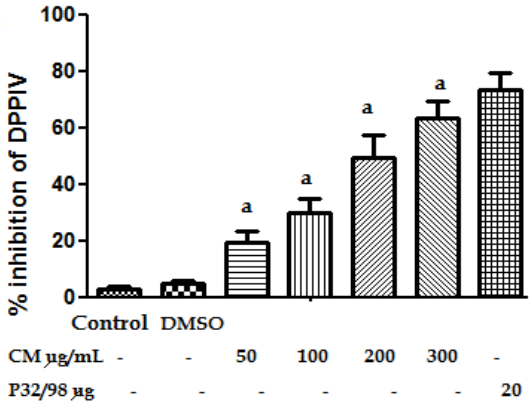

Fig 2. Effect of di□erent concentrations of CM on inhibition of DPPIV enzyme in comparison to the DMSO control and the commercially inhibitor (P32/98). Results are represented as mean ± SD (n=4). [a]p value<0.05 versus standard.

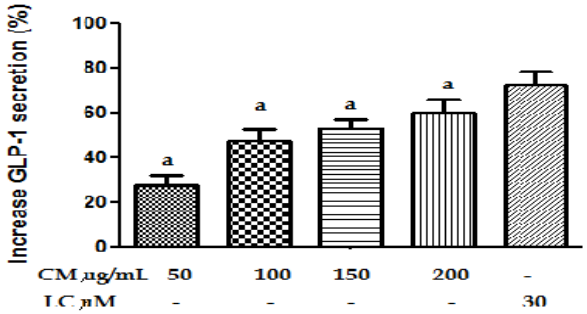

Fig 3. Values shown are means ± SD (n = 3). Values of [a]p <0.05 versus LC standard. Effect of CM on GLP-1 secretion in media from GLUTag cells at different concentrations (50, 100, 150 and 200 µg/L.

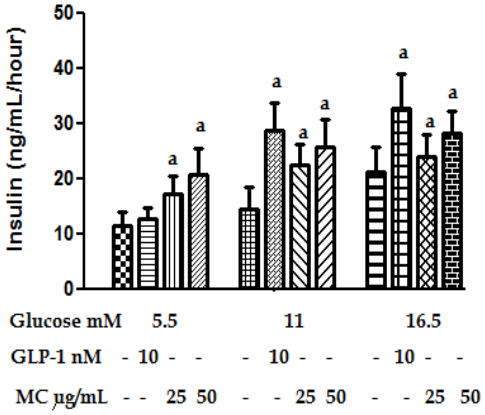

Fig 4. Glucose-stimulated insulin secretion from INS-1 cells cultured at 5.5, 11 and 16.5 mM for five days. GLP-1 (10 nM) was taken as positive control. Data shown are means ± SD (n = 3). Values of [a]p <0.05 versus the same glucose concentrations without MC cultured for five days.