(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 May 2012 (18.05.2012)

PCT

(10) International Publication Number
WO 2012/061931 A1

(51) International Patent Classification:
A61K 31/7048 (2006.01) *A61P 5/50* (2006.01)
A61P 3/10 (2006.01) *C07H 17/04* (2006.01)
A61P 39/00 (2006.01)

(21) International Application Number:
PCT/CA2011/001246

(22) International Filing Date:
10 November 2011 (10.11.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/412,966 12 November 2010 (12.11.2010) US

(71) Applicant *(for all designated States except US)*: **NATIONAL RESEARCH COUNCIL OF CANADA** [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WANG, Yanwen** [CA/CA]; 35 Gates Drive, Charlottetown, Prince Edward Island C1E 1R8 (CA). **ZHANG, Junzeng** [CA/CA]; 42 Scotch Pine Ter., Halifax, Nova Scotia B3S 1E2 (CA). **ZIDICHOUSKI, Jeffrey** [CA/CA]; 23 Nottingham Avenue, Charlottetown, Prince Edward Island C1A 8T4 (CA). **LIU, Jianhui** [CN/CN]; 26 Guangdian Road, Nan'an District, Chongqing, 400067 (CN). **YIN, Fei** [CN/CN]; 26 Guangdian Road, Nan'an District, Chongqing, 400067 (CN).

(74) Agents: **PATENAUDE, Sonia** et al.; National Research Council of Canada, IP Portfolio Management, 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: USE OF GENIPOSIDE IN DIABETES PREVENTION

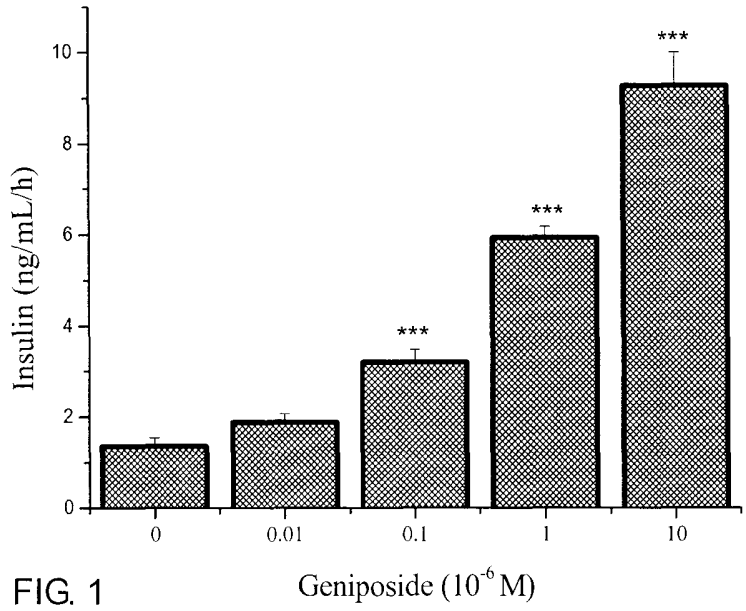


FIG. 1

(57) Abstract: The present invention relates to the use of a natural compound in diabetes prevention. More specifically, the invention relates to the use of geniposide as a pancreatic beta cell protectant for diabetes prevention and treatment.

WO 2012/061931 A1

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

USE OF GENIPOSIDE IN DIABETES PREVENTION

FIELD OF THE INVENTION

The present invention relates to the use of a natural compound in diabetes prevention. More specifically, the invention relates to the use of a natural compound as a pancreatic beta cell protectant for diabetes prevention and treatment.

BACKGROUND OF THE INVENTION

Diabetes (hyperglycemia) is due to either insulin resistance or deficiency of insulin secretion (Kahn, 2003; Gerich, 2003). In parallel with the increasing prevalence of obesity, diabetes has achieved epidemic status (Diabetes Care, 1997; Harris et al, 1998; MMWR, 2003; Wild et al, 2004; Larsson et al, 2006). The incidence of Type 2 diabetes, which highly correlates with diet and lifestyle (Kahn, 2003; Soodini et al, 2006), has particularly increased. According to the American Diabetes Association diagnostic criteria (Diabetes Care, 1997), the prevalence of diagnosed and undiagnosed diabetes in the United States in adults aged 40-74 years increased by 48% in between 1976 and 1994 (Harris et al, 1998). More recent data indicate that the prevalence of diabetes (diagnosed and undiagnosed) in the total population of adults of 20 years and older was 8.6% in 1999-2000. The prevalence of diabetes in Canada has increased from 4.3% in 1997-1998 to 5.5% in 2004-2005 (Public Health Agency of Canada). Diabetes is not only increasing at an alarming rate in North America, it has become a global epidemic. Based on available information and the trend of diabetes development in the past decades, it is estimated that the global prevalence of diabetes (including type 1 and type 2) in adults aged 20 years and over will increase by 39% by 2030. The prevalence is higher in developed countries than in developing countries.

Type 2 diabetes is a disease that previously occurred predominantly in adults. However, its prevalence has grown to become a health problem in children and adolescents, which is thought to be associated with the increasing incidence of obesity in this demographic (Larsson et al, 2006). Excess weight plays a critical role in the etiology of Type 2 diabetes mellitus, which is an important risk factor for premature coronary heart disease and stroke, as well as the main cause of kidney failure, limb amputations, and new-onset blindness in adults.

In the early stage of Type 2 diabetes, increased insulin secretion gradually exhausts the pancreatic beta cells, which leads to insulin deficiency. Prolonged high glucose stimulation to beta cells, due to peripheral insulin resistance may play an important role in the development of diabetes. Another contributing factor is the damage to beta cells caused by amyloid

polypeptide (hIAPP) aggregation, glucotoxicity, and lipotoxicity – the latter are collectively named glucolipotoxicity. In particular, hIAPP aggregation has been shown to cause beta cell toxicity and apoptosis (Zraika et al, 2010). Glucolipotoxicity is related to the oxidative damage of β cells, which in turn is considered to be highly associated with the development of diabetes.

Diabetes, especially Type 2, is highly associated with poor lifestyle choices and eating habits. Maintaining or changing one's habits to align with healthier lifestyle and nutritional habits are primary approaches for the treatment and prevention of diabetes. Other approaches to managing hyperglycemia include: protecting, preserving, or regenerating pancreatic beta cell mass and improving insulin secretion (Gallwitz, 2010; Yki-Jarvinen, 2004); inhibiting the intestinal breakdown of carbohydrates and thus to decrease or slow glucose absorption (Elhayany et al, 2010); and increasing peripheral tissue insulin sensitivity.

For example, hyperglycemia may be managed via an increase of glucose clearance (uptake) in the peripheral tissues such as muscle, liver, and adipose tissues (Yki-Jarvinen, 2004; Nakano et al, 2010; Nam et al, 2010). Among the drugs used in the treatment of diabetes is an insulin-sensitizing drug family that includes rosiglitazone; these compounds mainly target PPAR-gamma, affecting fat cell differentiation and insulin signalling (Yki-Jarvinen, 2004). The main disadvantage with this type of anti-diabetic drug is the increased risk of heart attack; this is of particular concern, since diabetics are already several times more likely to suffer cardiac arrest (Israili, 2009). Another significant side effect of this class of compounds is weight gain, which in turn worsens insulin resistance in the long term. If used long-term, rosiglitazone results in loss of insulin efficiency, and patients eventually must utilize commercially-available insulin (Israili, 2009). Another medication targeted to patients with Type 2 diabetes is acarbose, which partially blocks the absorption of carbohydrates in the small intestine; this produces GI-related side effects including stomach pain, and flatulence (Krentz 2006).

Thus, currently available over-the-counter or prescription drugs are not optimally effective, may have low efficacies, or lead to side effects that prevent long-term use. Moreover, products to prevent the onset or progression of diabetes, especially for Type 2 diabetes, are lacking. As a result, there is a strong demand to develop effective and safe products to treat diabetes, as well as for the prevention or protection against diabetes development and progression.

SUMMARY OF THE INVENTION

The present invention relates to the use of a natural compound in diabetes prevention. More specifically, the invention relates to the use of a natural compound as a pancreatic beta cell protectant for diabetes prevention and treatment.

The present invention provides a method of modulating insulin secretion in pancreatic cells, comprising contacting the cells with an effective amount of geniposide. Additionally, the present invention includes a method of modulating insulin secretion in pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

The present invention also provides a method of modulating glucose consumption in cells, comprising contacting the cells with an effective amount of geniposide. Additionally, the present invention includes a method of modulating glucose consumption in cells, comprising administering an effective amount of geniposide to a subject in need thereof.

Also provided is a method of increasing beta cell viability or reducing cytotoxicity of hIAPP and free fatty acid, comprising contacting the cells with an effective amount of geniposide. Additionally, the present invention includes a method of increasing beta cell viability or reducing cytotoxicity of hIAPP and free fatty acid, comprising administering an effective amount of geniposide to a subject in need thereof.

The present invention further provides a method of reducing cytotoxicity of human islet amyloid polypeptide (hIAPP) and/or its aggregations in pancreatic cells, comprising contacting the cells with an effective amount of geniposide. Additionally, the present invention includes a method of reducing cytotoxicity of human islet amyloid polypeptide (hIAPP) and/or its aggregations in pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

The present invention further provides a method of reducing glucotoxicity and lipotoxicity (herein collectively referred to as glucolipotoxicity) in pancreatic cells, comprising contacting the cells with an effective amount of geniposide. Additionally, the present invention includes a method of reducing glucotoxicity and lipotoxicity in pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

Additionally, a method of protecting pancreatic cells is provided. The method comprises contacting the cells with an effective amount of geniposide; alternatively, the method may comprise administering an effective amount of geniposide to a subject in need thereof.

The present invention provides a method of preventing pancreatic beta cell exhaustion, comprising administering an effective amount of geniposide to a subject in need thereof; alternatively, the method may comprise administering an effective amount of geniposide to a subject in need thereof.

The present invention also provides a method of preventing or treating diabetes, comprising administering an effective amount of geniposide to a subject in need thereof; alternatively, the method may comprise administering an effective amount of geniposide to a subject in need thereof.

Diabetes research and related product development efforts have been heavily focused on understanding the etiology of diabetes and ways or treatments that can reverse or improve diabetic conditions. However, prevention of diabetes development or protection of key organs and tissues involved in glucose metabolism is equally important in improving diabetic conditions. The present invention focuses on the preservation or protection of pancreatic beta cell mass and function against detrimental factors. Preservation of the pancreatic beta cells when insulin-receptor resistance or high circulating glucose conditions occur, instead of taxing beta cells to maximize its insulin secretion in order to lower blood glucose, is a particular goal of the present invention. Continuously high levels of blood glucose stimulate pancreatic beta cells to secrete insulin, whose function is to lower blood glucose. Consequently, this process compromises beta cells, which can lead to cell death, or diminution or complete loss of beta-cell function. Preventing pancreatic beta cells from over-producing and releasing insulin during periods of very high blood glucose challenges could protect beta cell mass and function. Thus, protection and preservation of beta cell function are an increasingly important strategy in diabetes prevention and treatment, particularly since evidence indicates beta cell function deterioration starts at pre-diabetes stage and is closely related to the progression of diabetes (DeFronzo, 2010; Marchetti et al, 2009; Robertson, 2009). Additionally, hyperglycemia can be detrimental to many peripheral tissues or organs (for example, pancreatic cells) via increased oxidative stress and other mechanisms.

It is presently shown that a natural compound, geniposide, provides comprehensive protection against high glucose stress on beta cells. As shown herein, geniposide induced insulin secretion in the presence of low to high concentrations of glucose (such as 5.5mM to 16.7mM) via regulating the uptake and utilization of glucose, including the production of ATP, in rat insulinoma beta cells. Interestingly, in the presence of a very high concentration of glucose (such as 33mM), geniposide reduces insulin secretion and decreased the uptake and utilization of glucose, compared to the stimulation of a very high glucose concentration alone. Thus, geniposide shows promise as an interventional target for protection against the onset of diabetes and its progression, due to its capacity to preserve pancreatic beta cells from quick exhaustion due to prolonged over-secretion of insulin due to high glucose burdens, from lipotoxicity or glucolipotoxicity, from the effects of human islet amyloid polypeptide (hIAPP) aggregation, and from damage due to oxidative stress. Geniposide can be used as a beta cell

protectant in humans who are at a high relative risk for developing Type 2 diabetes, or diagnosed to have pre- or early diabetes.

Additional aspects and advantages of the present invention will be apparent in view of the following description. The detailed description and examples, while indicating preferred embodiments of the invention, are given by way of illustration only, as various changes and modifications within the scope of the invention will become apparent to those skilled in the art in light of the teachings of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features of the invention will now be described by way of example, with reference to the appended drawings, wherein:

FIGURE 1 is a bar graph showing that geniposide induces insulin secretion in a dose-dependent manner in the absence of glucose in INS-1 cells. The INS-1 cells with passage number of 68-73 were seeded at a density of 5×10^5 cells/well on 12-well-plates. The next day, the cells were washed twice with KRBH buffer and starved for 2 hours in KRBH buffer. Then, the cells were cultured in fresh KRBH buffer containing different concentrations of geniposide (0, 0.01, 0.1, 1.0, or 10 μM). Following one-hour incubation, the medium was collected and analyzed for insulin concentration using the commercial ELISA kits following the manufacturer's instructions (Linco Research Inc. Missouri, USA). Data are means ± SD from four independent experiments. *** $P < 0.001$ vs control.

FIGURE 2 shows the effect of geniposide on the biphasic glucose-stimulated insulin secretion in INS-1 cells. INS-1 cells were seeded onto 12-well-plates and incubated for 24 hours. The cells were washed twice with fresh KRBH and starved for two hours in KRBH buffer. The cells were treated with 10 μM geniposide in KRBH buffer in the presence or absence of 5.5 mM glucose (G5.5) for 20 min. The buffer was collected in a 30-second intervals to determine the insulin secretion using the commercial ELISA kits following the manufacturer's instructions (Linco Research Inc. Missouri, USA). Data are means of two independent experiments.

FIGURE 3 illustrates that geniposide potentiates glucose-stimulated insulin secretion in INS-1 cells in the absence and presence of glucose ranging from low to high concentrations. INS-1 cells were seeded onto 12-well-plates and incubated for 24 hours. The cells were washed twice with fresh KRBH and starved for 2 hours in KRBH buffer. KRBH containing different concentrations glucose (0, 5.5, 11 or 16.7 mM) with or without 10 μM geniposide. Following a one-hour incubation, the buffer was collected and analyzed for insulin concentration using

commercial ELISA kits following the manufacturer's instructions (Linco Research Inc. Missouri, USA). Data are means ± SD of six independent experiments. ** $P < 0.01$, *** $P < 0.001$ vs control.

FIGURE 4 is a bar graph showing that geniposide decreases glucose-stimulated insulin secretion in the presence of a very high concentration of glucose. INS-1E cells were seeded in 12-well-plates and incubated for 24 hours. The cells were washed twice with fresh KRBH and starved for 2 hours in KRBH buffer. The cells were then incubated for one hour in KRBH containing 5.5mM or 33 mM of glucose with or without 10 μM geniposide. Following a one-hour incubation, the buffer was collected and analyzed for insulin concentrations using commercial kits according the manufacturer's instructions (Linco Research Inc. Missouri, USA). Data are means ± SD from six independent experiments. * $P < 0.05$ vs control.

FIGURE 5 is a bar graph illustrating the effects of geniposide on the utilization of glucose in INS-1E cells. After INS-1E cells were washed twice with PBS, they were starved for 2 hours in the absence or presence of 10 μM of geniposide (Gen), and then cultured in KRBH buffer comprising 5.5 or 33 mM glucose for 20 min. The buffer was collected and measured for glucose concentration using an enzymatic method (GenZyme Diagnostics PEI Inc., Charlottetown, PEI, Canada). Data are means ± SD from six independent experiments. *p < 0.05 vs the group of 33 mM alone.

Figure 6 is a bar graph showing the effect of geniposide on ATP production induced by 5.5, 11, and 33 mM glucose in INS-1 cells. INS-1 cells were treated with 10 μM geniposide and different glucose concentrations for 1 hour. The cells were collected to determine ATP content. Data are means ± SD from three independent experiments. **p < 0.01, *p < 0.05 compared to treatment using the same glucose concentration in the absence of geniposide.

FIGURE 7 is a bar graph showing that geniposide is not cytotoxic in INS-1E pancreatic beta cell line at indicated concentrations. INS-1E cells were seeded onto 12-well-plates and incubated for 24 hours. The cells were then incubated with 0, 0.1, 1, 10, 100, and 1000 μM geniposide for an additional 24 hours. Cell viability was determined using the MTT assay. Data are means ± SD from three independent experiments.

Figure 8 is a bar graph showing that geniposide reverses human islet amyloid polypeptide (hIAPP)-induced cell damage or cytotoxicity in INS-1E pancreatic beta cell line. After INS-1E cells were treated with geniposide at 0, 0.1, 1.0, 10, or 100 μM for 2 hours, the cells were incubated with 0 or 2 μM hIAPP for another 24 hours. Cell viability was determined using the

MTT assay. Data are means ± SD from 3 independent experiments * $p < 0.05$, ** $p < 0.01$ vs. hIAPP alone.

FIGURE 9 is a bar graph showing that palmitate (PA) induces the decrease of cell viability in a dose-dependent manner in INS-1 pancreatic beta cell line. After INS-1 cells were incubated with 0.2, 0.3, or 0.4 mM palmitate for 18 hours, cell viability was determined using the MTT assay. Data are means ± SD from 6 separate experiments. ** $p < 0.01$ compared to control cells incubated with 0.5% BSA alone.

FIGURE 10 illustrates the effect of geniposide on the viability of palmitate-stressed INS-1 cells. INS-1 cells were pre-treated with geniposide (0, 1.0, 10, or 100 μM) for 2 hours and subsequently cultured for 7 hours in RPMI medium in the presence or absence of 0.4 mM palmitate (PA). Data are expressed as means ± SD from 4 separate experiments. $^{\#}p < 0.05$ compared with 0.5% BSA alone; * $p < 0.05$ compared to cells treated with 0.4 mM palmitate and 0.5% BSA.

FIGURE 11 shows the effects of geniposide on the expression of HO-1 and UCP-2 proteins INS-1 cells treated with normal glucose (FIGURE 11A) and high glucose (FIGURE 11B) media, respectively. After treatment with 0, 0.1, 1 or 10 μM geniposide for 24 hours, the cells were washed once with PBS, and the level of UCP-2 and HO-1 proteins were detected with western blotting. Values are the means ± SD from three separate experiments. $^{**}P < 0.01$ vs control.

FIGURE 12 shows the structure of geniposide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of a natural compound in diabetes prevention. More specifically, the invention relates to the use of a natural compound as a pancreatic beta cell protectant for diabetes prevention and treatment.

The present invention is directed to the use of a natural compound, geniposide, to provide comprehensive protection against high glucose stress, lipotoxicity, and toxicity of human islet amyloid polypeptide (hIAPP) aggregation on pancreatic beta cells. Results described herein indicate that geniposide may preserve pancreatic beta cells from exhaustion due to prolonged insulin secretion due to high glucose burdens, from lipotoxicity or glucolipotoxicity, from the effects of human islet amyloid polypeptide (hIAPP) aggregation, and from damage due to oxidative stress.

Geniposide is a natural compound whose structure is shown in Figure 12. This iridoid glycoside can be obtained from the fruit of *Gardenia jasminoides* Ellis, which is a popular Chinese herb used to treat hepatic disorders, acute conjunctivitis, inflammatory diseases, vitiligo vulgaris and hematuria (Wang et al, 1992; Kang et al, 1997; Toyoda et al, 2008; Koo et al, 2006). Until now, various bioactivities of geniposide have been identified in numerous studies, including modulation on antioxidative enzymes (Kang et al, 1997), antitumor (Koo et al, 2004; Kim et al, 2007), anti-inflammation (Koo et al, 2006), etc. Studies have suggested that geniposide activates glutathione S-transferase (GST) by the induction of GST M1 and GST M2 subunits involving the transcription and phosphorylation of MEK-1 signaling in rat hepatocytes (Kuo et al, 2005), and showed the radical scavenging activity (Wang et al, 2007). In particular, geniposide has been demonstrated to protect rat hippocampal slice culture against oxygen and glucose deprivation-induced neuronal cell death (Usta et al, 2006). Other work has shown that geniposide is an agonist for glucagon-like peptide 1 receptor (GLP-1R), which induced the neuronal differentiation of PC12 cells and prevented PC12 cells from oxidative stress (Liu et al, 2006; Liu et al, 2007; Liu et al, 2009). However, the protective effects of geniposide against various stresses, cell damages, or death in pancreatic beta cells and their underlying mechanisms are unknown.

The geniposide used in the methods described herein may be provided in pure form, as an extract or a bioactive fragment thereof, or in a composition.

As used herein, the term "geniposide" is intended to include compositions or formulations comprising geniposide. Formulations or compositions comprising geniposide may also include other compounds that may be useful for preserving pancreatic beta cells from exhaustion, reducing human islet amyloid polypeptide (hIAPP) aggregation, reducing lipotoxicity, or gluoclipotoxicity, and/or reducing oxidative stress; such additional compounds may be natural or pharmaceutical compounds. Such other compounds may include, but are not limited to analogues of geniposide (such as genipin, penta-acetyl-geniposide, aucubin, gentiopicroside, catalpol, etc), metformin, or alpha-glucosidase inhibitiors (such as acarbose). For example, and without wishing to be limiting in any manner, the composition or formulation may comprise a mixture (or "cocktail") of geniposide and such natural or pharmaceutical compounds; such a mixture may provide a composition with increased potency. Compositions also include extracts comprising geniposide; such extracts may include buffers, salts, and other components as listed below.

Compositions or formulations comprising geniposide may comprise a pharmaceutically acceptable carrier, diluent or excipient (or "pharmacologically acceptable ingredient"). The carrier, diluent or excipient may be any suitable carrier, and must be compatible with other

ingredients in the composition, with the method of delivery of the composition, and must not be deleterious to the recipient of the composition. The composition may be in any suitable form; for example, the composition may be provided in liquid form, suspension form, powder form (for example, lyophilised), capsule or tablet form. For example, and without wishing to be limiting, when the composition is provided in suspension form, the carrier may comprise water, saline, a suitable buffer, or additives to improve solubility and/or stability; reconstitution to produce the suspension is effected in a buffer at a suitable pH. In a specific, non-limiting example, the pharmaceutically acceptable carrier may be saline. Dry powders may also include additives to improve stability and/or carriers to increase bulk/volume; for example, and without wishing to be limiting, the dry powder composition may comprise sucrose or trehalose. In a specific, non-limiting example, the composition may be so formulated as to deliver the compound to the gastrointestinal tract of the subject, or in a time-release manner. Thus, the composition may comprise encapsulation, time-release, or other suitable technologies for delivery of geniposide. It would be within the competency of a person of skill in the art to prepare suitable compositions comprising the present compounds.

For example, and without wishing to be limiting in any manner, pharmacologically acceptable ingredients for nutraceutical and/or pharmaceutical compositions, include anti-adherents, binders (e.g. starches, sugars, cellulose, hydroxypropyl cellulose, ethyl cellulose, lactose, xylitol, sorbitol and maltitol), coatings (e.g. cellulose, synthetic polymers, corn protein zein and other polysaccharides), disintegrants (e.g. starch, cellulose, cross-linked polyvinyl pyrrolidone, sodium starch glycolate and sodium carboxymethyl cellulose), fillers/diluents (e.g. water, plant cellulose, dibasic calcium phosphate, vegetable fats and oils, lactose, sucrose, glucose, mannitol, sorbitol and calcium carbonate), flavors and colors, glidants, lubricants (e.g. talc, silica, vegetable stearin, magnesium stearate and stearic acid), preservatives (e.g. vitamin A, vitamin E, vitamin C, selenium, cysteine, methionine, citric acid, sodium citrate, methyl paraben and propyl paraben), antioxidants, sorbents, sweeteners, and mixtures thereof.

The present invention provides a method of modulating insulin secretion in pancreatic beta cells, comprising contacting the cells with an effective amount of geniposide or administering an effective amount of geniposide to a subject in need thereof. Geniposide may control insulin secretion in a dose-dependent manner, and the effect may differ based on glucose concentration. For example, and without wishing to be limiting in any manner, geniposide may increase insulin secretion in the presence of low, normal, or moderately high glucose concentrations, but may inhibit or block further increase of insulin secretion in the presence of high or very high glucose concentrations such as during the postprandial period. (As would be understood by a person of skill in the art, glucose concentrations are relative to the normal

glucose concentration for beta cells: pre-meal plasma glucose of 5 to 7.2 mM and a post-meal glucose levels of less than 10mM according to the recommendations of the American diabstes association). This type of effect is beneficial, as a prolonged increase in insulin secretion can exhaust pancreatic beta cells. Thus by inhibiting insulin secretion at high or very high glucose levels, geniposide preserves pancreatic beta cells and their functions, and avoids the development of insulin resistance.

Also provided is a method of modulating glucose consumption or utilization in beta cells, comprising contacting the cells with an effective amount of geniposide or administering an effective amount of geniposide to a subject in need thereof. The effect of geniposide may differ based on glucose concentration.

The present invention further provides a method of increasing viability or reducing cytotoxicity of hIAPP and free fatty acid (such as palmitate) in pancreatic beta cells, comprising contacting the cells with an effective amount of geniposide or administering an effective amount of geniposide to a subject in need thereof. The presence of geniposide may decrease the beta cell damage induced by human islet amyloid polypeptide (hIAPP), with increasing the cell viability. Additionally, the presence of geniposide may decrease the lipotoxicity or glucolipotoxicity in pancreatic beta cells, suggesting that pretreatment with geniposide might be helpful the treatment or preventing the oxidative injury of beta cell induced by high glucose or/ and high lipid.

Additionally, the present invention provides a method of reducing human islet amyloid polypeptide (hIAPP) aggregation in pancreatic cells, comprising contacting the cells with an effective amount of geniposide or administering an effective amount of geniposide to a subject in need thereof. Aggregation of hIAPP in beta cells damages the cells and prevents proper functioning. Geniposide was shown (see Examples below) to be a mild to moderate inhibitor of hIAPP aggregation.

The present invention further provides a method of reducing glucotoxicity and lipotoxicity (collectively referred to as glucolipotoxicity) in pancreatic cells, comprising contacting the cells with an effective amount of geniposide or administering an effective amount of geniposide to a subject in need thereof.

In view of the capacity of geniposide to preserve pancreatic beta cells from exhaustion due to prolonged insulin secretion or excessive or over release due to high glucose burdens, from the effects of hIAPP aggregation, from lipotoxicity or gluoclipitoxicity, and from damage due to oxidative stress, the present invention provides a method of protecting pancreatic cells,

comprising contacting the cells with an effective amount of geniposide or administering an effective amount of geniposide to a subject in need thereof.

In its capacity to preserve pancreatic beta cells from exhaustion, reduce hIAPP aggregation, and reduce oxidative stress, the present invention also provides a method of preventing pancreatic beta cell exhaustion, comprising administering an effective amount of geniposide to a subject in need thereof or administering an effective amount of geniposide to a subject in need thereof.

There is also provided a method for treatment or prevention of diabetes, comprising administering an effective amount of geniposide to a subject in need thereof.

Geniposide and/or formulations comprising geniposide may be delivered by any suitable route of administration known in the art. For example, and without wishing to be limiting in any manner, the compounds of the present invention or composition thereof may be delivered systemically (orally, nasally, parentally, intravenously, etc.) or may be delivered to the gastrointestinal tract. In a specific, non-limiting example, the compositions of the present invention are administered orally or parentally. Those of skill in the art would be familiar with such methods of delivery.

Geniposide and/or compositions comprising geniposide may be used or administered in any suitable amount or dosage ("effective amount") that results in the desired effect. As would be recognized by one of skill in the art, the particular amount or dosage will vary based on the specific compound, the route of administration, the severity of the subject's diabetes, and the specifics of the subject. However, a suitable amount or dosage may be, but is not limited to the range of about 0.01 to 750 mg/kg of bodyweight per day; for example, the suitable amount may be 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 mg/kg of bodyweight per day, or any amount there between.

The present invention further provides a kit or commercial packaging comprising geniposide and/or compositions comprising geniposide, along with instructions for use in the methods described herein. The kit or commercial package may optionally include other compounds known to be useful for preserving pancreatic beta cells from exhaustion, reducing human islet amyloid polypeptide (hIAPP) aggregation, reducing lipotoxicity or glucolipotoxicity, and/or reducing oxidative stress (such as those described above), buffers, and/or administration aids (such as syringes, needles, antiseptic wipes, etc). For example, when the compound or composition is provided in powder form, the kit may include a buffer for dissolution or suspension of the compound or composition, as well as a suitable container for mixing the two

components. The kit or commercial package may also include instructions for use in the methods described herein.

The present invention will be further illustrated in the following examples. However, it is to be understood that these examples are for illustrative purposes only and should not be used to limit the scope of the present invention in any manner.

Example 1: Effect of geniposide on insulin secretion in INS-1 or INS-1E cells

The effect of geniposide on insulin secretion in rat insulinoma INS-1 or INS-1E cells exposed to various glucose concentrations including high and very high glucose concentrations was evaluated.

INS-1 or INS-1E cell line was grown in RPMI-1640 medium supplemented with 10 mM HEPES, 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 µM mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified incubator with 5% CO_2 at 37°C. The cells with passage number of 68-73 were seeded on 12-well plates and cultured for 24 hours in the same medium. The cells were washed two times with KRBH buffer (25 mM Hepes, 120 mM NaCl, 4.6 mM KCl, 1.9 mM $CaCl_2$, 1 mM $MgSO_4$, and 1.2 mM KH_2PO_4, pH 7.4) and starved for 2 hours in KRBH buffer containing 1 mM glucose and 0.5% BSA in the presence of 100 µM geniposide. For all Examples, geniposide was obtained from Sichuan Dicotyledonous Bio-tech Co., Ltd (Sichuan, China; purity over 99.5%, UR20060421) and dissolved in steriled 1 × PBS to obtain a 10 mM stock solution. The buffer was changed and the cells were challenged with different concentrations of glucose ranging from 5.5 to 33 mM. Following one-hour incubation (Figs. 1, 3, and 4) or different time points (Fig.2), the medium was collected and insulin concentrations was anaylzed using commercial ELISA kits (Linco Research Inc. Missouri, USA) according to manufacturer's instructions.

Results show that geniposide increased insulin secretion in a dose-dependent manner in rat insulinoma INS-1 cells (Fig. 1), and at a concentration of 10 µM, tended to increase insulin secretion in INS-1E cells in the presence of low glucose (Figs. 2 and 3). In contrast, when the cells were cultured at a very high glucose concentration of 33 mM, geniposide inhibited insulin secretion compared to cells treated with 33mM glucose alone (Fig. 4). Geniposide increases insulin secretion in INS-1E cells in a dose-dependent manner as compared to cells that were not treated with geniposide; however the effect depended on the glucose levels in the culture medium (Fig. 4).

Example 2: Effect of geniposide on glucose uptake in INS-1E cells

The effect of geniposide on glucose uptake in rat insulinoma INS-1E cells in the presence of different glucose concentrations was evaluated.

INS-1E cell line was grown in RPMI-1640 medium supplemented with 10 mM HEPES, 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 μM mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 at 37°C. Cells with passage number of 68-73 were seeded on 12-well plates and cultured for 24 hours. Cells were starved in serum-free RPMI medium with 10 μM geniposide, respectively, for 2 hours. The cells were washed twice with PBS and cultured in KRBH buffer (25 mM Hepes, 120 mM NaCl, 4.6 mM KCl, 1.9 mM $CaCl_2$, 1 mM $MgSO_4$, and 1.2 mM KH_2PO_4, pH 7.4) containing 1 mM glucose and 0.5% BSA. Cells were then incubated with 5.5 or 33 mM glucose for 20 min at 37°C; the buffer was collected and measured for glucose concentration with the enzymatic method using the reagents purchased from GenZyme Diagnostics PEI Inc. (Charlotttetown, PEI, Canada).

Results showed that geniposide treatment resulted in a higher glucose consumption/utilization when 5.5 mM glucose was added at the beginning, (Fig. 5). In contrast, when a very high glucose concentration such as 33 mM was added to the medium, geniposide reduced glucose consumption.

Example 3 Geniposide and glucose-induced ATP production in pancreatic beta cells

Insulin secretion requires ATP; therefore, ATP levels in INS-1E cells are positively associated with insulin secretion. To determine the effect of geniposide on ATP production, INS-1E cells were cultured, passaged, starved and incubated as described above. Following the 20-min incubation, cell lysates were collected by centrifuging at 15,800 g for 15 min. The supernatants were collected to determine ATP levels using a commercial kit (FLAA-1KT, Sigma-Aldrich Canada Ltd., Oakville, On). Results demonstrated treatment with 10 μM geniposide increased ATP levels in INS-1E cells in the presence of 5.5 and 11 mM mM glucose in the medium, respectively, but decreased ATP levels in the presence of 33 mM glucose (Fig. 6).

These results indicated that geniposide decreased ATP production in response to a very high glucose challenge, possibly by inhibiting glucose uptake in INS-1E cells. Without wishing to be bound by theory, this effect may ultimately lead to the inhibition of over-release/secretion of insulin and consequently protect beta cells from dysfunction or death.

Example 4: Geniposide and human islet amyloid polypeptide (hIAPP)-induced damage of INS-1E cells

The effect of geniposide on hIAPP-induced damage of INS-1E cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay.

hIAPP, also referred to as human islet amyloid polypeptide or human islet amylin (Am), was prepared by dissolving 1 mg of hIAPP peptide in 1 ml hexafluoroisopropanol (HFIP), and sonicated in a water-bath for 10 min. The solution was aliquoted (100 µl) into microcentrifuge tubes to obtain 0.1 mg/tube (stocks). The tubes were kept in the dark at room temperature for 5-24 hours. HFIP was removed by evaporation under N_2, leaving a thin transparent peptide film on the internal surface of the tube. Tubes were stored at -80°C. Prior to use, the hIAPP was dissolved in 10 mM PBS (pH 7.4) containing 1 % HFIP to make a final solution at a concentration of 1 mM.

INS-1E rat insulinoma cells were grown in RPMI-1640 medium supplemented with 10 mM HEPES, 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 µM mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. A hIAPP solution of 250 µM (dissolved 0.1 mg hIAPP into 100 µL 10 mM PBS, pH 7.4) was freshly prepared. The rat insulenoma INS-1E beta cells were seeded on 24-well microplates at a density of 3 x 10^5 cells/well. After 24 hr, they were washed once with PBS and cultured with phenol-free red RPMI 1640 medium comprising 10 mM HEPES, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 µM mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin . The cells were pre-treated with 0, 0.1, 1, 10, 100, or 1000 µM geniposide for 2 hr. After 2 hr, cells were co-incubated with 2 µM hIAPP for another 24 hours. Following geniposide pre-treatment or hIAPP co-incubation, cells were then were incubated with MTT at 0.5 mg/mL for 2-4 hours at 37°C; formazan salt generated by viable cells as a result of conversion of MTT was dissolved in DMSO and the absorbance was measured at 570 nm, with 630 nm as reference, to evaluate the protective effect of geniposide on INS-1E cells from hIAPP-induced cell damage.

Results showed that geniposide was not cytotoxic to INS-1E cells in the dosage range of 0.1–1000 µM (Fig. 7). The cytotoxicity of hIAPP was significantly reduced in the presence of geniposide ranging from 0.1 to 100 µM, as evidenced by markedly increased cell viability with increasing geniposide concentration up to 100 µM (Fig. 8).

Example 5: Geniposide and palmitate-induced toxicity in INS-1 cells

The toxicity of free fatty acids, such as palmitate, in pancreatic beta cells was tested in INS-1 rat insulinoma cell line.

INS-1 rat insulinoma cells were grown in RPMI-1640 medium supplemented with 10 mM HEPES, 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 μM mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. After INS-1 cells were incubated with palmitate at concentrations of 0.2, 0.4, and 0.4 mM for 18 hours. Or, INS-1 cells were pretreated with geniposide at indicated concentrations for 2 hours and subsequently cultured for 7 hours in RPMI medium in the presence or absence of 0.4 mM palmitate (PA). The cell viability was determined with the MTT assay.

The results demonstrate that palmitate induced cytotoxicity in INS-1 cells in a dose-dependent manner (Fig. 9). However, geniposide reversed the toxicity of palmitate in a dose-dependent manner (Fig. 10).

Example 6: Effect of geniposide on oxidative stress

The effect of geniposide on oxidative stress was determined in a rat insulenoma INS-1 cell line.

INS-1 rat insulinoma cells were grown in RPMI-1640 medium supplemented with 10 mM HEPES, 10% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 μM mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. Cells were seeded on 6-well microplates at a density of 5×10^5 cells/well. After 24 hours of incubation, the media was replaced with fresh media comprising 11 mM or 25 mM glucose. The cells were treated with 0, 0.1, 1.0 or 10 μM geniposide for 24 hours. The cells were washed once with PBS and 1× RIPA was added to extract the total protein. Equal amount of proteins were separated on a 10% SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% non-fat milk then the expression of UCP-2 and HO-1 was probed with primary antibodies (Cell Signal Technology Co Ltd) specific for each protein.

Results are shown in Figures 11A and 11B. Geniposide increased the protein expression of the anti-oxidant marker, HO-1, particularly in the presence of high concentrations of glucose. Furthermore, geniposide decreased UCP-2 protein expression when challenged with high glucose. These results indicate that geniposide could alleviate the oxidative stress caused by

high glucose by regulating the expression of anti-oxidative protein HO-1 and energy metabolic protein UCP-2 in INS cells.

The embodiments and examples described herein are illustrative and are not meant to limit the scope of the invention as claimed. Variations of the foregoing embodiments, including alternatives, modifications and equivalents, are intended by the inventors to be encompassed by the claims. Furthermore, the discussed combination of features might not be necessary for the inventive solution.

REFERENCES

All patents, patent applications and publications referred to herein and throughout the application are hereby incorporated by reference.

DeFronzo, R. A. Overview of newer agents: where treatment is going. The American Journal of Medicine 2010, 123, S38-S48.

Elhayany, A.; Lustman, A.; Abel, R.; Attal-Singer, J.; Vinker, S., A low carbohydrate Mediterranean diet improves cardiovascular risk factors and diabetes control among overweight patients with type 2 diabetes mellitus: a 1-year prospective randomized intervention study. Diabetes Obes Metab 2010,12, (3), 204-9.

Gallwitz, B., The evolving place of incretin-based therapies in type 2 diabetes. Pediatr Nephrol. 2010, 25 (7), 1207-17.

Harris, M. I.; Flegal, K. M.; Cowie, C. C.; Eberhardt, M. S.; Goldstein, D. E.; Little, R. R.; Wiedmeyer, H. M.; Byrd-Holt, D. D., Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994. Diabetes Care 1998, 21, (4), 518-24.Prevalence of diabetes and impaired fasting glucose in adults--United States, 1999-2000. MMWR Morb Mortal Wkly Rep 2003, 52, (35), 833-7.

Israili, Z. H., Advances in the Treatment of Type 2 Diabetes Mellitus. Am J Ther 2009.

Kahn, S. E., The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003, 46, (1), 3-19.Gerich, J. E., Contributions of insulin-resistance and insulin-secretory defects to the pathogenesis of type 2 diabetes mellitus. Mayo Clin Proc 2003, 78, (4), 447-56.

Kang JJ, Wang HW, Liu TY, Chen YC, Ueng TH. Modulation of cytochrome P-450-dependent monooxygenases, glutathione and glutathione S-transferase in rat liver by geniposide from Gardenia jasminoides. Food Chem Toxicol, 1997, 35, 957-65.

Kim CY, Kim J. Preparative isolation and purification of geniposide from gardenia fruits by centrifugal partition chromatography. Phytochem Anal, 2007, 18, 115-7.

Koo HJ, Lee S, Shin KH, Kim BC, Lim CJ, Park EH. Geniposide, an anti-angiogenic compound from the fruits of Gardenia jasminoides. Planta Med, 2004, 70, 467-9.

Koo HJ, Lim KH, Jung HJ, Park EH. Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. J Ethnopharmacol, 2006, 103, 496-500.

Krentz, A. J., Comparative safety of newer oral antidiabetic drugs. Expert Opin Drug Saf 2006, 5, (6), 827-34.

Kuo WH, Chou FP, Young SC, Chang YC, Wang CJ. Geniposide activates GSH S-transferase by the induction of GST M1 and GST M2 subunits involving the transcription and phosphorylation of MEK-1 signaling in rat hepatocytes. Toxicol Appl Pharmacol, 2005, 208, 155-62.

Larsson, S. C.; Wolk, A., Epidemiology of Obesity and Diabegtes. In: Obesity and Diabetes. Edited by Mantzoros, C.S. Human Press, Totowa, New Jersey, 2006, pp15-38. 2006.

Liu J, Yin F, Zheng X, Jing J, Hu Y. Geniposide, a novel agonist for GLP-1 receptor, prevents PC12 cells from oxidative damage via MAP kinase pathway. Neurochem Int, 2007, 51, 361-9.

Liu J, Zheng X, Yin F, Hu Y, Guo L, Deng X, Chen G, Jiajia J, Zhang H. Neurotrophic property of geniposide for inducing the neuronal differentiation of PC12 cells. Int J Dev Neurosci, 2006, 24, 419-24.

Liu JH, Yin F, Guo LX, Deng XH, Hu YH. Neuroprotection of geniposide against hydrogen peroxide induced PC12 cells injury: involvement of PI3 kinase signal pathway. Acta Pharmacol Sin, 2009, 30, 159-65.

Marchetti, P., Lupi, R., Guerra, S. D., Bugliani, M., D'Aleo, V., Occhipinti, M., Boggi, U., Marselli, L., Masini, M. Goals of treatment for type 2 diabetes: beta cell preservation for glycemic control, Diabetes Care 2009, 32, S178-S193.

Nakano, K.; Hasegawa, G.; Fukui, M.; Yamasaki, M.; Ishihara, K.; Takashima, T.; Kitagawa, Y.; Fujinami, A.; Ohta, M.; Hara, H.; Adachi, T.; Ogata, M.; Obayashi, H.; Nakamura, N., Effect of pioglitazone on various parameters of insulin resistance including lipoprotein subclass according to particle size by a gel-permeation high-performance liquid chromatography in newly diagnosed patients with type 2 diabetes. Endocr J. 2010, 57 (5), 4230-30.

Nam, J. S.; Nam, J. Y.; Yoo, J. S.; Cho, M.; Park, J. S.; Ahn, C. W.; Cha, B. S.; Lee, E. J.; Lim, S. K.; Kim, K. R.; Lee, H. C., The effect of rosiglitazone on insulin sensitivity and mid-thigh low-density muscle in patients with Type 2 diabetes. Diabet Med 2010, 27, (1), 30-6.

Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997, 20, (7), 1183-97.

Robertson, R. P. Beta-cell deterioration during diabetes: what's in the gun? Trends in Endocrinology and Metabolism 2009, 20, 388-393.

Soodini, G. R.; Hamdy, O., Pathophysiology of Diabetes in Obesity. In: Obesity and Diabetes. Edited by Mantzoros, C.S., Humana Press, Totowa, New Jersey, pp117-125, 2006.

Toyoda K, Okitsu T, Yamane S, Uonaga T, Liu X, Harada N, Uemoto S, Seino Y, Inagaki N. GLP-1 receptor signaling protects pancreatic beta cells in intraportal islet transplant by inhibiting apoptosis. Biochem Biophys Res Commun, 2008, 367, 793-8.

usta B, Baggio LL, Estall JL, Koehler JA, Holland DP, Li H, Pipeleers D, Ling Z, Drucker DJ. GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. Cell Metab, 2006, 4, 391-406.

Wang Q, Chen K, Liu R, Zhao F, Gupta S, Zhang N, Prud'homme GJ. Novel GLP-1 fusion chimera as potent long acting GLP-1 receptor agonist. PLoS One, 2007, 5, e12734.

Wang SW, Lai CY, Wang CJ. Inhibitory effect of geniposide on aflatoxin B1-induced DNA repair synthesis in primary cultured rat hepatocytes. Cancer Lett, 1992, 65, 133-7.

Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H., Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004, 27, (5), 1047-53.

Yki-Jarvinen, H., Thiazolidinediones. N Engl J Med 2004, 351, (11), 1106-18.

Zraika, S., Hull, R. L., Verchere, C. B., Clark, A., Potter, K. J., Fraser, P. E., Raleigh, D. P., and Kahn, S. E. Toxic oligomers and islet beta cell death: guilty by association or convicted by circumstantial evidence? Diabetologia 2010, 53, 1046-1056.

5

CLAIMS:

1. A method of modulating insulin secretion in pancreatic cells, comprising contacting the cells with an effective amount of geniposide.

2. A method of modulating glucose consumption in cells, comprising contacting the cells with an effective amount of geniposide.

3. A method of increasing beta cell viability, comprising contacting the cells with an effective amount of geniposide.

4. A method of reducing cytotoxicity, comprising contacting the cells with an effective amount of geniposide.

5. A method of reducing cytoxicity induced by human islet amyloid polypeptide (hIAPP) aggregations in pancreatic cells, comprising contacting the cells with an effective amount of geniposide.

6. A method of reducing glucotoxicity and lipotoxicity in pancreatic cells, comprising contacting the cells with an effective amount of geniposide

7. A method of protecting pancreatic cells, comprising contacting the cells with an effective amount of geniposide.

8. A method of modulating insulin secretion in pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

9. A method of modulating glucose consumption in cells, comprising administering an effective amount of geniposide to a subject in need thereof.

10. A method of increasing beta cell viability, comprising administering an effective amount of geniposide to a subject in need thereof.

11. A method of reducing cytotoxicity, comprising administering an effective amount of geniposide to a subject in need thereof.

12. A method of reducing cytoxicity induced by human islet amyloid polypeptide (hIAPP) aggregations in pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

13. A method of reducing glucotoxicity and lipotoxicity in pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

14. A method of protecting pancreatic cells, comprising administering an effective amount of geniposide to a subject in need thereof.

15. A method of preventing pancreatic beta cell exhaustion, comprising administering an effective amount of geniposide to a subject in need thereof.

16. A method of preventing or treating diabetes, comprising administering an effective amount of geniposide to a subject in need thereof.

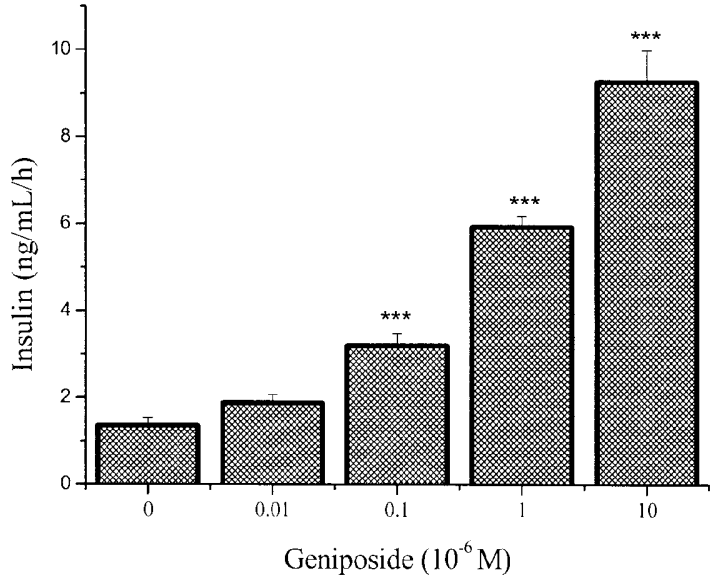

Insulin (ng/mL/h)
10
8
6
4
2
0
***
***
***
0
0.01
0.1
1
10
Geniposide (10^{-6} M)

FIG. 1

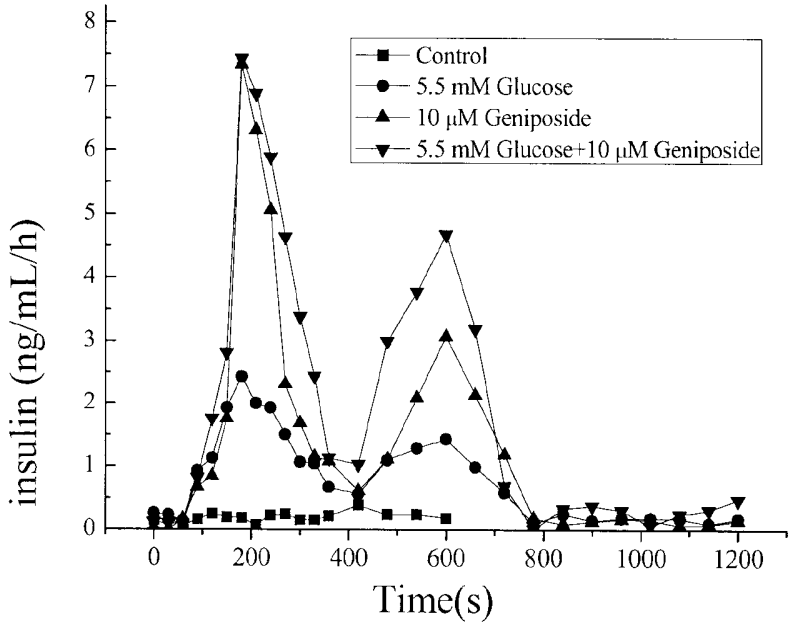

insulin (ng/mL/h)
8
7
6
5
4
3
2
1
0
Control
5.5 mM Glucose
10 μM Geniposide
5.5 mM Glucose+10 μM Geniposide
0
200
400
600
800
1000
1200
Time(s)

FIG. 2

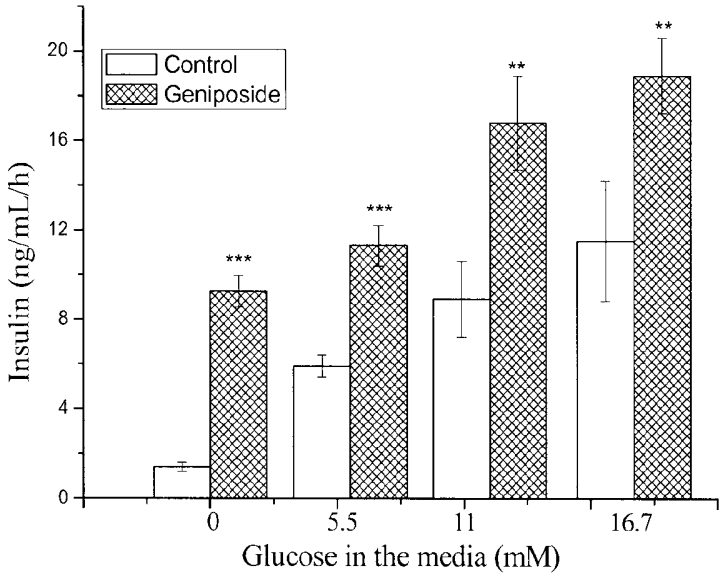

Control
Geniposide
Insulin (ng/mL/h)
Glucose in the media (mM)
0
5.5
11
16.7
***
***
**
**

FIG. 3

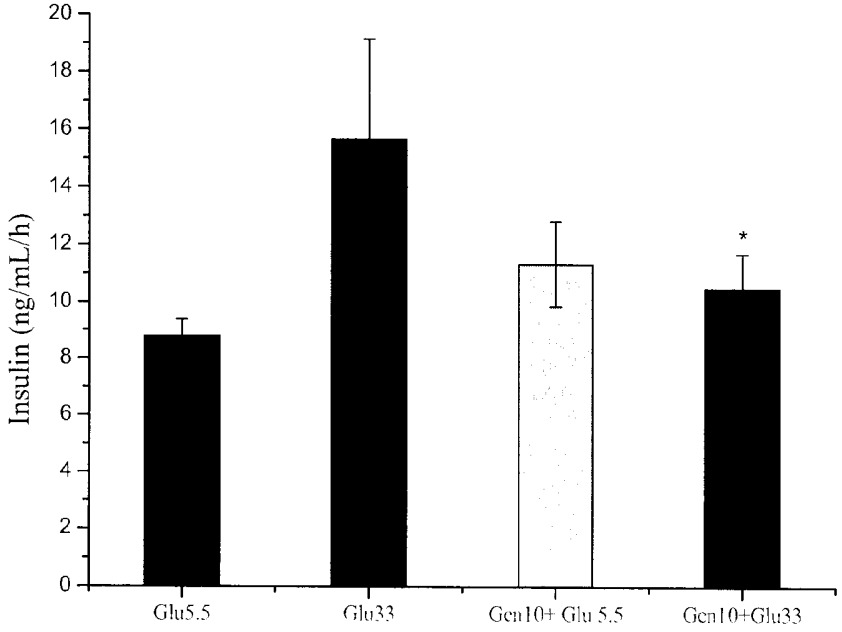

Insulin (ng/mL/h)
Glu5.5
Glu33
Gen10+ Glu 5.5
Gen10+Glu33
*

FIG. 4

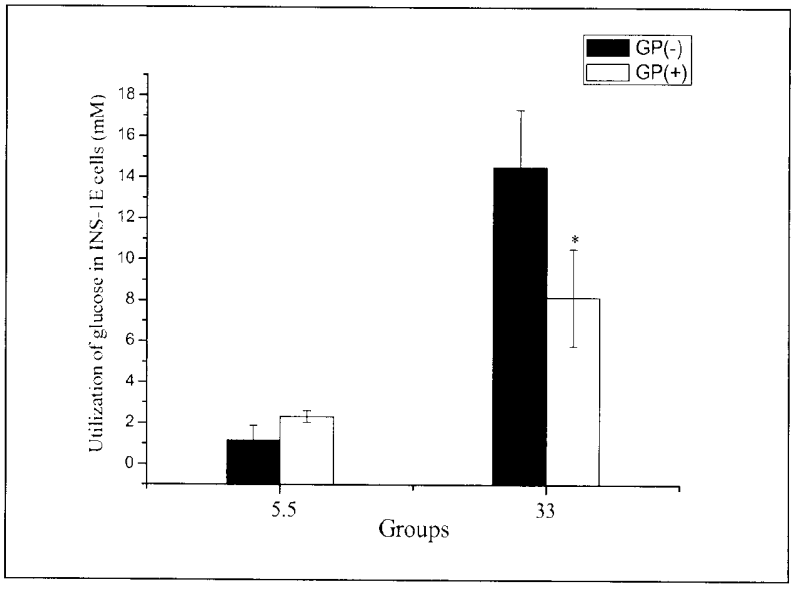

GP(-)
GP(+)
Utilization of glucose in INS-1E cells (mM)
0
2
4
6
8
10
12
14
16
18
*
5.5
33
Groups

FIG. 5

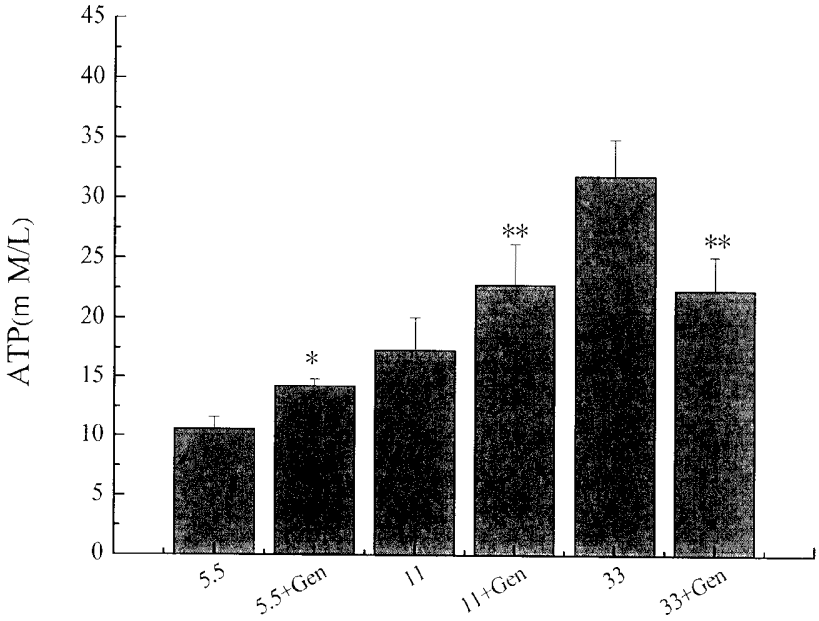

ATP(m M/L)
0
5
10
15
20
25
30
35
40
45
*
**
**
5.5
5.5+Gen
11
11+Gen
33
33+Gen

FIG. 6

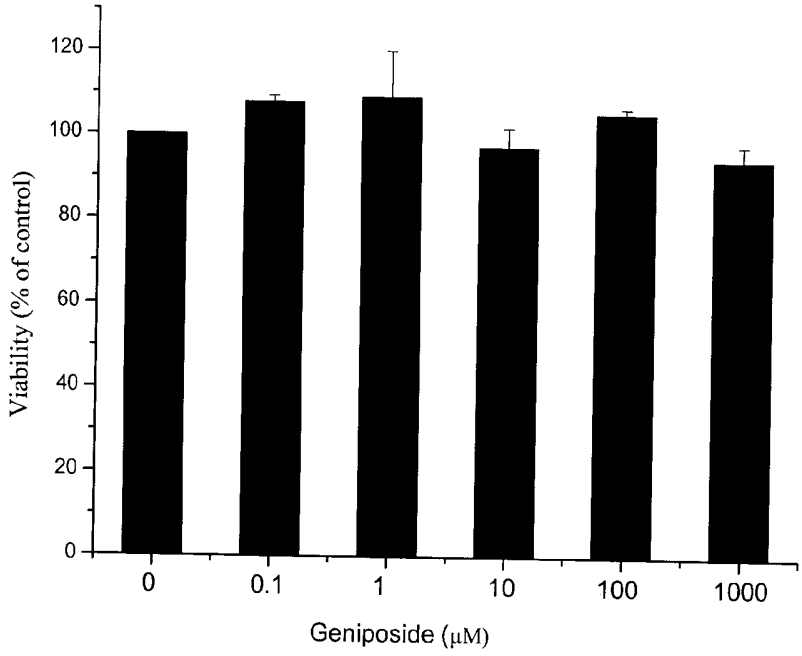

Viability (% of control)
120
100
80
60
40
20
0
0
0.1
1
10
100
1000
Geniposide (μM)

FIG. 7

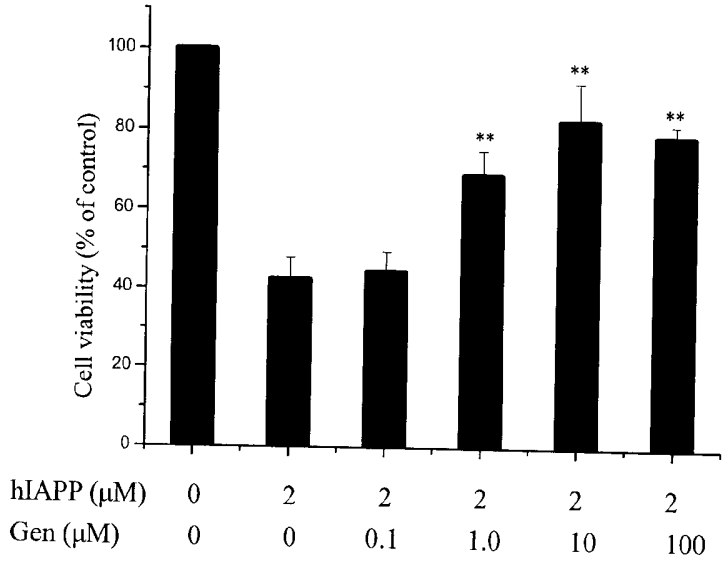

Cell viability (% of control)
100
80
60
40
20
0
**
**
**
hIAPP (μM) 0 2 2 2 2 2
Gen (μM) 0 0 0.1 1.0 10 100

FIG. 8

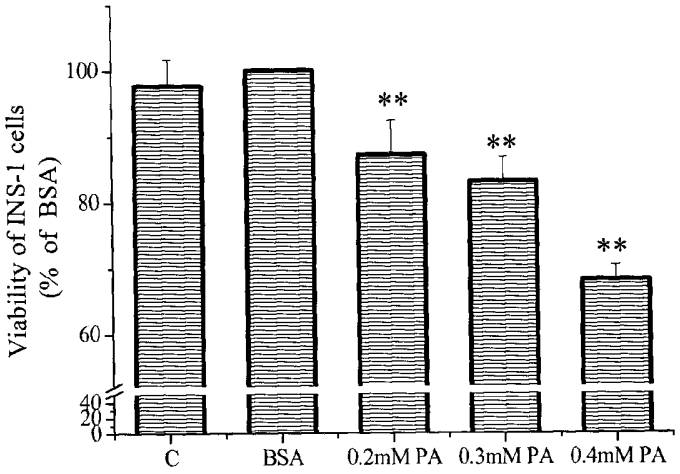

Viability of INS-1 cells
(% of BSA)
100
80
60
40
20
0
**
**
**
C
BSA
0.2mM PA
0.3mM PA
0.4mM PA

FIG. 9

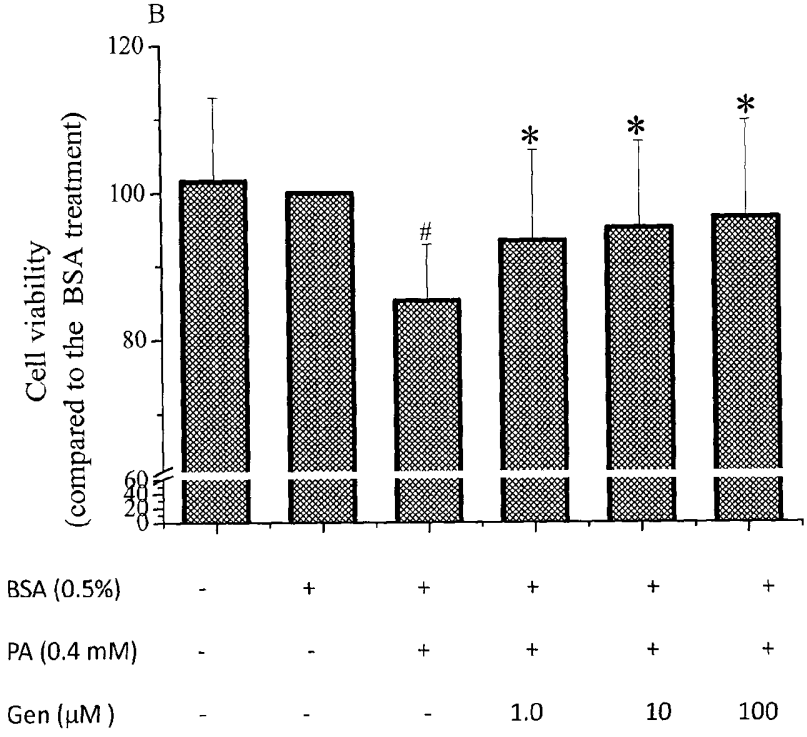
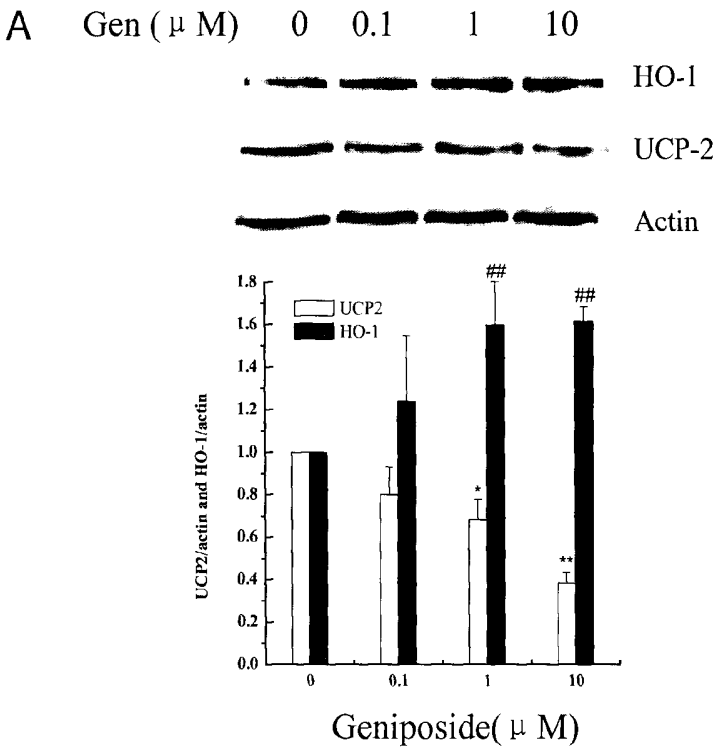

B
Cell viability
(compared to the BSA treatment)
120
100
80
60
40
20
0
#
*
*
*
BSA (0.5%) - + + + + +
PA (0.4 mM) - - + + + +
Gen (μM) - - - 1.0 10 100

FIG. 10


A
Gen (μ M) 0 0.1 1 10
HO-1
UCP-2
Actin
UCP2
HO-1
UCP2/actin and HO-1/actin
Geniposide(μ M)

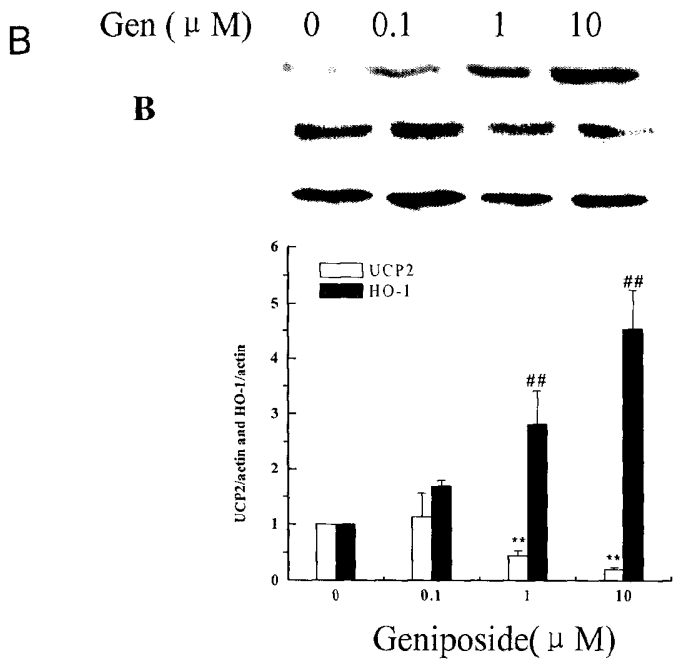

B
Gen (μ M) 0 0.1 1 10
B
UCP2
HO-1
UCP2/actin and HO-1/actin
Geniposide(μ M)

FIG. 11

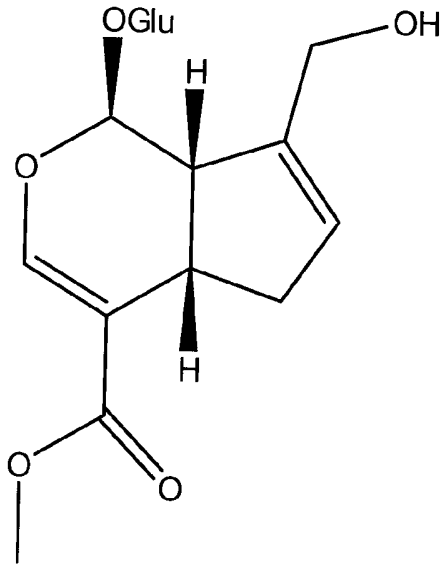


FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/001246

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 31/7048*** (2006.01), ***A61P 3/10*** (2006.01), ***A61P 39/00*** (2006.01), ***A61P 5/50*** (2006.01), ***C07H 17/04*** (2006.01).

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/7048 (2006.01), ***A61P 3/10*** (2006.01), ***A61P 39/00*** (2006.01), ***A61P 5/50*** (2006.01), ***C07H 17/04*** (2006.01).

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
A61K 31/70

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: EPODOC, TotalPatent, Canadian Patent Database, PubMed, Scopus .
Keywords: Diabet*, insulin, glucose, pancrea*, beta+cell | geniposide OR RN=24512-63-8.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	KOJIMA et al. Biol. Pharm. Bull., **34** (10), pp 1613-1618 October 2011 (10-2011) (Epub: 11 July 2011 (11-07-2011)) "Preventive Effect of Geniposide on Metabolic Disease Status in Spontaneously Obese Type 2 Diabetic Mice and Free Fatty Acid-Treated HepG2 Cells." [ISSN: 0918-6158] http://www.jstage.jst.go.jp/article/bpb/34/10/1613/_pdf (See Abstract; Tables 1 & 2; Discussion)	1 - 16
X	WU et al. Acta Pharmacol. Sin., **30** (2), pp 202-208. Feb 2009 (02-2009) "Effect of geniposide, a hypoglycemic glucoside, on hepatic regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin." [doi: 10.1038/aps.2008.17], [ISSN: 1671-4083] http://www.nature.com/aps/journal/v30/n2/pdf/aps200817a.pdf (See Abstract; p 203; p205; Table 2; Discussion)	1 - 16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 2012 (26-01-2012)	24 February 2012 (24-02-2012)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer C. Bourque (819) 934-3596

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/001246

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIMURA *et al.*, Chem Pharm Bull (Tokyo), **30** (12), pp 4444-4447. December 1982 (12-1982) "Effects of geniposide isolated from Gardenia jasminoides on metabolic alterations in high sugar diet-fed rats." [ISSN: 0009-2363] http://www.journalarchive.jst.go.jp/jnlpdf.php?cdjournal=cpb1958&cdvol=30&noissue=12&startpage=4444&lang=en&from=jnltoc (See Abstract; Tables I & II; Discussion)	1 - 16
X	WANG *et al.*, Acta Pharmacol. Sin., **31** (8), pp. 953 - 962. Aug 2010 (08-2010) "Geniposide inhibits high glucose-induced cell adhesion through the NF-kappaB signaling pathway in human umbilical vein endothelial cells." doi: 10.1038/aps.2010.83 [ISSN: 1671-4083 http://www.nature.com/aps/journal/v31/n8/full/aps201083a.html (See Abstract; p 957 - 958)	1 - 16
A	Zhang *et al.*, Cell Metabolism, **3** (6), pp. 417- 427. 6 June 2006 (06-06-2006) "Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced β cell dysfunction in isolated pancreatic islets." http://dx.doi.org/10.1016/j.cmet.2006.04.010, http://www.sciencedirect.com/science/article/pii/S155041310600129X (See Abstract; p421-422 and Discussion)	
A	US2006/0148724 A1 Zhang *et al.* 06 July 2006 (06-07-2006) GENIPIN DERIVATIVES AND USES THEREOF (See paragraphs 0178 - 0180; 0183 -0184; 0187 - 0192)	
A	MARCHETTI *et al.* Diabetes Care., **32** (Suppl 2), pp. S178-83. Nov 2009 (11-2009) "Goals of treatment for type 2 diabetes: beta-cell preservation for glycemic control."doi: 10.2337/dc09-S306 [ISSN: 0149-5992] http://care.diabetesjournals.org/content/32/suppl_2/S178.full.pdf+html (See entire document)	
A	ROBERTSON R.P. Trends Endocrinol. Metab., **20**(8):388-393. October 2009 (10-2009) "Beta-cell deterioration during diabetes: what's in the gun?" [ISSN: 1043-2760] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2756315/pdf/nihms-142088.pdf (See entire document)	

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1 - 16

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1 - 16 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search (See Rule 39.1 (iv)). However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in said claims.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2011/001246

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2006148724A1	06 July 2006 (06-07-2006)	US2006148724A1	06 July 2006 (06-07-2006)
		US2008009450A9	10 January 2008 (10-01-2008)
		US7649014B2	19 January 2010 (19-01-2010)
		US2010168160A1	01 July 2010 (01-07-2010)
		US8093288B2	10 January 2012 (10-01-2012)
		WO2004089926A2	21 October 2004 (21-10-2004)
		WO2004089926A8	20 January 2005 (20-01-2005)
		WO2004089926A3	30 June 2005 (30-06-2005)