Sargassum sagamianum extract protects INS-1 pancreatic β cells against high glucose-induced apoptosis

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Abstract This study investigated the protective effects of *Sargassum sagamianum* extract (SSE) on INS-1 pancreatic β cells against high glucose-induced oxidative stress and apoptosis. Treatment with glucose at high concentrations (30 mM) caused β cell apoptosis, whereas treatment with SSE protected the β cells from high glucose-induced damage, by recovering the cell viability. Treatment with SSE at concentrations of 10–100 μ g/mL decreased lipid peroxidation and intracellular reactive oxygen species and nitric oxide levels, and increased cell viability and insulin secretion in high glucose pretreated INS-1 cells in a dose-dependent manner. Moreover, SSE treatment significantly reduced the expression of pro-apoptotic Bax, cytochrome *c*, caspase-3, and caspase-9, while the expression of anti-apoptotic Bcl-2 increased. The type of cell death was examined by annexin V/propidium iodide staining, which revealed that SSE treatment markedly reduced high glucose-induced apoptosis. These findings suggest that SSE could be useful as a functional food, protecting pancreatic β cells against high glucose-induced oxidative stress and apoptosis.

Keywords Apoptosis · INS-1 · Pancreatic β cells · *Sargassum sagamianum*

Introduction

Type 2 diabetes is characterized by hyperglycemia, resulting from insulin resistance and a defect in insulin secretion. Pancreatic β cells, which secrete insulin, are sensitive to oxidative stress induced by high glucose levels that can lead to glucotoxicity and eventually, β cell death with type 2 diabetes (Paneni et al. 2013; Donath and Halban 2004). Both the functional defects and decreased β cell mass contribute to β cell failure in type 2 diabetes, with apoptosis constituting the main form of β cell death. Although the precise mechanism underlying β cell death is not known, one of the major factors contributing to cell death is oxidative stress induced by hyperglycemia. Hyperglycemic conditions result in the production of large quantities of reactive oxygen species (ROS) in the mitochondria and a decrease in the activities of antioxidant enzymes, causing oxidative stress. Oxidative stress leads to β cell glucotoxicity, resulting in cellular dysfunction and apoptosis (Robertson et al. 2007). Thus, to prevent pancreatic β cell dysfunction and apoptosis, it is necessary to use substances that reduce the oxidative stress and apoptosis caused by hyperglycemia (Talchai et al. 2012; Kaneto et al. 1999).

Seaweeds contain a wide array of bioactive substances with diverse health benefits that counteract metabolic syndromes and diabetes. *Sargassum sagamianum*, an edible brown alga, was harvested from the coastal areas of Korea and Japan. *Sargassum sagamianum* is used in Korea for food such as

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ingredients of bread (Kim et al. 2011), or as a treatment for oral diseases (Choi et al. 2012), but SSE is not well known in the world. There are some studies for *sargassum sagamianum* extract (SSE) has been shown to be effective in the treatment of anti-inflammatory (Kim et al. 2013) and antibacterial (Horie et al. 2008) effects. SSE contains bioactive compounds, such as plastoquinones, phlorotannins, and farnesylacetone derivatives, which exhibit cholinesterase inhibitory activity in Alzheimer's disease (Choi et al. 2007). Also, SSE alleviates postprandial hyperglycemia in diabetic mice (Lee and Han 2018). However, the effects of SSE on pancreatic β cell dysfunction and survival have not been examined. Thus, this study investigated the protective effects of SSE against high glucose-induced apoptosis in INS-1 β cells.

Materials and methods

Materials

Sargassum sagamianum was collected along the coast of Jeju Island in Korea. The sample was washed three times with tap water to remove salt, epiphytes, and sand attached to the surface, and then carefully rinsed with fresh water and freeze-dried. The dried sample was extracted three times with ten volumes of 80% ethanol for 12 h at room temperature. The filtrate was then vacuum-evaporated to obtain the extract. SSE was thoroughly freeze-dried and stored in a deep freezer (-80°C).

Cell culture

INS-1 pancreatic β cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS), streptomycin (100 $\mu\text{g}/\text{mL}$), and penicillin (100 units/mL) at 37°C in a humidified atmosphere containing 5% CO_2 . Cells were cultured in 5.5 or 30 mM glucose. The normal glucose level was represented by 5.5 mM, and 30 mM represented a high glucose level.

Cell viability assay

Cell viability was assessed by the colorimetric 3-(4,5-dime-thylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT) assay, based on the conversion of MTT to MTT-formazan by mitochondrial enzymes. Cells (2×10^4 cells/well) were pre-incubated with glucose (5.5 or 30 mM) for 48 h, and then incubated with or without 10, 25, 50, and 100 $\mu\text{g}/\text{mL}$ SSE. Next, 100 μL of MTT solution (Sigma-Aldrich Co., St. Louis, MO, USA) was added to each well of a 96-well culture plate, and the solutions were incubated for 4 h at 37°C , before removal of the MTT-containing medium. In the viable cells, the incorporated formazan crystals were solubilized with 100 μL of dimethyl sulfoxide (Bio Basic Inc., NY, USA), and the absorbance of each well was read at 540 nm using a microplate reader.

Intracellular ROS level determination

Intracellular ROS levels were measured using a dichlorofluorescein (DCF) assay. 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) can be deacetylated in cells. Then, it can quantitatively react with intracellular radicals for conversion to the fluorescent product DCF, which is retained within the cells. Therefore, DCF-DA was used to evaluate the generation of ROS during oxidative damage. Cells (2×10^4 cells/well) were seeded in 96-well black plates and pre-incubated with glucose (5.5 and 30 mM) for 48 h, and then incubated with or without 10, 25, 50, and 100 $\mu\text{g}/\text{mL}$ SSE for 24 h. The medium was removed, and the cells were washed with phosphate-buffered saline (PBS) and incubated with 5 μM DCF-DA for 30 min at room temperature. Fluorescence was measured using a fluorescence plate reader (LS-5B; Perkin-Elmer, Waltham, MA, USA).

Lipid peroxidation assay

Lipid peroxidation was measured based on the production of thiobarbituric acid reactive substances (TBARS). Cells in 96-well plates (2×10^4 cells/well) were pre-incubated with glucose (5.5 and 30 mM) for 48 h at 37°C and 5% CO_2 . Next, the cells, with or without 10, 25, 50, and 100 $\mu\text{g}/\text{mL}$ SSE, were further incubated for 24 h. A sample of 200 μL was mixed with 400 μL of the TBARS solution and boiled at 95°C for 20 min. The absorbance at 532 nm was measured, and the TBARS concentrations were extrapolated from the 1,1,3,3-tetraethoxypropane standard curve. The thiobarbituric acid value was

expressed as the equivalent nanomoles of malondialdehyde (MDA).

Determination of the nitric oxide (NO) levels

Cells (2×10^4 cells/well) in a 96-well plate were pre-incubated with 5.5 and 30 mM glucose for 48 h. Then, the cells were incubated with or without 10, 25, 50, and 100 $\mu\text{g/mL}$ SSE for 24 h. The accumulated NO levels in the supernatant were assessed using the Griess method. Thereafter, 50 μL of each culture supernatant was mixed with an equal volume of the Griess reagent [0.1% *N*-(1-naphthyl)-ethylene-diamine and 1% sulfanilamide in 5% phosphoric acid], and the mixture was incubated at room temperature for 10 min. The absorbance of the resulting solution was measured at 550 nm using a microplate reader. A series of known concentrations of sodium nitrite was used as the standard.

Glucose-stimulated insulin secretion

Cells (2×10^5 cells/well) in a 6-well plate were pre-incubated with 5.5 or 30 mM glucose for 48 h, and then incubated with or without SSE for 48 h. Thereafter, the medium was removed, and the cells were washed with PBS, before fresh medium was added. After 5 h of incubation, the cells were stimulated with Krebs–Ringer buffer [119 mM NaCl, 4.75 mM KCl, 2.54 mM CaCl_2 , 1.2 mM MgSO_4 , 5 mM NaHCO_3 , and 20 mM HEPES, pH 7.4], containing 5 or 25 mM glucose, for 60 min at 37 °C, and then, the medium was collected to investigate the insulin secretion. Insulin secretion was measured by a rat/mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Lingo Research, Lingo, MO, USA).

Flow cytometric assessment of apoptosis

Cell death, due to apoptosis, was quantified for treatment with 5.5 or 30 mM glucose in the presence or absence of SSE, by Annexin-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining. The cell suspension (100 μL) was incubated with 5 μL of annexin-V and 5 μL of PI. Cells were incubated in the dark for 15 min in an ice bath. Thereafter, 400 μL of binding buffer was added to the cell suspension; the suspensions were then promptly examined using a flow cytometer (FACS caliber and CellQuest

software; Becton–Dickinson, San Jose, CA, USA). The number of apoptotic cells were expressed as a percentage of the total number of cells.

Western blot analysis

Cells in T-75 were pre-incubated with 5.5 or 30 mM glucose for 48 h, and then incubated with or without the indicated concentration of SSE for 48 h. Cell lysates were prepared with ice-cold lysis buffer [250 mM NaCl and 25 mM Tris–HCl (pH 7.5), 1% (v/v) NP-40, 1 mM DTT, 1 mM PMSF, and a protease inhibitor cocktail (10 $\mu\text{g/mL}$ of aprotinin and 1 $\mu\text{g/mL}$ of leupeptin)]. The cell lysates were washed by concentrations and centrifuged at $10,000 \times g$ for 15 min at 4 °C. Total and nuclear protein contents were determined using the Bio-Rad protein kit with BSA as the standard. Lysates containing 20 μg protein were subjected to electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gels, and the resolved proteins were transferred onto nitrocellulose membranes. The membranes were incubated with antibodies raised against Bax, Bcl-2, cytochrome *c*, caspase-9, caspase-3, and β -actin, in Tris-buffered saline with tween-20 (TTBS) (25 mM Tris–HCl, 137 mM NaCl, and 0.1% Tween 20; pH 7.4), containing 5% milk for 1 h. The membrane was washed once with TBS-T and incubated with secondary antibodies. Signals were developed using an ECL western blotting detection kit (Bio-Rad) and exposed to X-ray film.

Data and statistical analysis

Data were represented as the mean $\pm$ standard deviation. Statistical analysis was performed using SAS software (SAS Institute, Inc., Cary, NC, USA). The experimental values were evaluated by one-way analysis of variance, followed by post hoc Duncan's multiple range tests. Differences between two groups were compared using *t*-tests.

Results

Cell viability

The effect of SSE on the cell viability of INS-1 β cells, treated with glucose, was examined using the MTT assay, as shown in Fig. 1. The cell viability was

Fig. 1 Effect of SSE on the viability of high glucose-treated INS-1 pancreatic β cells. Cells in 96-well plates (2×10^4 cells/well) were pre-incubated with 5.5 or 30 mM glucose for 48 h, and subsequently incubated for 48 h in the presence or absence of 10, 25, 50, and 100 $\mu\text{g}/\text{mL}$ SSE. Glucose concentration of 5.5 mM was representative of normal glucose levels, while the 30-mM glucose treatment represented high glucose level conditions. Each value is expressed as the mean $\pm$ SD ($n = 3$). ^{a-c}Values with different letters are significantly different with $p < 0.05$, as analyzed by Duncan's multiple range test

significantly ($p < 0.05$) reduced to 37.87% by pre-treating with 30 mM glucose in INS-1 β cells, but SSE treatment recovered the cellular damage caused by 30 mM glucose in a dose-dependent manner. In particular, treatment with 100 $\mu\text{g}/\text{mL}$ SSE resulted in a significant ($p < 0.05$) increase in the cell viability to 68.27%.

Intracellular ROS generation

As shown in Fig. 2, the generation of intracellular ROS in INS-1 β cells was significantly ($p < 0.05$) increased to 196.65% after pretreatment with 30 mM glucose, compared to that in cells pretreated with 5.5 mM glucose. However, SSE treatment significantly ($p < 0.05$) decreased the level of ROS induced by treatment with 30 mM glucose in the INS-1 β cells. Treatment with 100 $\mu\text{g}/\text{mL}$ SSE resulted in a significant ($p < 0.05$) decrease in the generation of ROS to 121.88%. Therefore, SSE decreased the ROS levels, which were elevated due to the presence of glucose at high concentrations.

Fig. 2 Effect of SSE on intracellular ROS generation in the high glucose-treated INS-1 pancreatic β cells. Cells in 96-well plates (2×10^4 cells/well) were pre-incubated with glucose and incubated in the absence or presence of SSE, as described in the legend of Fig. 1. Each value is expressed as the mean $\pm$ SD ($n = 3$). ^{a-e}Values with different letters are significantly different with $p < 0.05$, as analyzed by Duncan's multiple range test

Fig. 3 Effect of SSE on TBARS generation in the high glucose-treated INS-1 pancreatic β cells. Cells in 96-well plates (2×10^4 cells/well) were pre-incubated with glucose and incubated in the absence or presence of SSE, as described in the legend of Fig. 1. Each value is expressed as the mean $\pm$ SD ($n = 3$). ^{a-e}Values with different letters are significantly different at $p < 0.05$, as analyzed by Duncan's multiple range test

Lipid peroxidation

Figure 3 shows the effect of SSE on lipid peroxidation in the high glucose-pretreated INS-1 β cells. In the INS-1 β cells, pretreated with 5.5 mM glucose, the lipid peroxidation level was 0.11 nmole MDA/mg protein, whereas cells pretreated with 30 mM glucose had 0.27 nmole MDA/mg protein. The cells treated with 100 $\mu\text{g}/\text{mL}$ SSE showed a significant ($p < 0.05$) decrease in lipid peroxides of 0.17 nmole MDA/mg

protein, indicating inhibition of lipid peroxide formation caused by the high glucose levels.

NO level

Figure 4 shows that pretreatment with 30 mM glucose significantly ($p < 0.05$) increased the NO level in INS-1 β cells, compared to that in cells pretreated with 5.5 mM glucose. However, SSE treatment decreased NO generation in a dose dependent manner. The NO level was 197.25% in the INS-1 β cells pretreated with 30 mM glucose, but treatment with 100 $\mu\text{g/mL}$ SSE in cells pretreated with high glucose concentration, resulted in a significantly ($p < 0.05$) reduced NO level (97.67%). SSE effectively inhibited NO generation induced by high glucose levels.

Apoptosis-related protein expression

To investigate whether SSE could suppress the expression of genes associated with high glucose-induced apoptosis, SSE at concentrations of 50 and 100 $\mu\text{g/mL}$ was added to INS-1 β cells pretreated with high glucose concentration. The level of the pro-apoptotic protein Bax increased more significantly ($p < 0.05$) in the cells pretreated with 30 mM glucose than that in cells pretreated with 5.5 mM glucose (Fig. 5), but treatment with 50 and 100 $\mu\text{g/mL}$ SSE significantly ($p < 0.05$) decreased Bax expression in the high glucose-pretreated cells. In addition,

Fig. 4 Effect of SSE on the NO level of the high glucose-treated INS-1 pancreatic β cells. Cells in 96-well plates (2×10^4 cells/well) were pre-incubated with glucose and incubated in the absence or presence of SSE, as described in the legend of Fig. 1. Each value is expressed as the mean $\pm$ SD ($n = 3$). ^{a–e}Values with different letters are significantly different at $p < 0.05$, as analyzed by Duncan's multiple range test

treatment with SSE significantly ($p < 0.05$) decreased the expression of caspase-9, caspase-3, and cytochrome *c* in the high glucose-pretreated cells, but significantly ($p < 0.05$) increased the expression of the anti-apoptotic protein, Bcl-2 in the high glucose-pretreated cells (Figs. 5, 6). Therefore, SSE treatment reduced pro-apoptotic protein expression and increased anti-apoptotic protein expression in the β cells damaged by high glucose levels.

Protective effects against apoptosis

In the flow cytometry analysis with annexin-V and PI double staining, the upper and lower right quadrants indicated the number of early and late apoptotic cells (Fig. 7). The number of apoptotic cells among the β cells pretreated with high glucose levels increased remarkably, compared to that among the normal glucose pretreated cells, but treatment with SSE reduced apoptosis in the high glucose-pretreated cells. In particular, treatment with 100 $\mu\text{g/mL}$ SSE resulted in a significantly decreased number of apoptotic cells.

Glucose-stimulated insulin secretion

Insulin secretion in the INS-1 β cells decreased significantly as a result of the high glucose pretreatment, compared to that in cells subjected to normal glucose pretreatment (Fig. 8). After removing the medium and washing the cells, the cells were treated with 5 and 25 mM glucose to measure the insulin secretion ability. The 25-mM glucose treatment resulted in more insulin secretion than the 5-mM glucose treatment. Treatment with 100 $\mu\text{g/mL}$ SSE significantly ($p < 0.05$) increased insulin secretion in the cells treated with 5 or 25 mM glucose, indicating that SSE increases insulin secretion in high glucose-pretreated INS-1 β cells.

Discussion

Pancreatic β cells play a major role in maintaining glucose homeostasis in the body by secreting insulin (Paolisso et al. 1990). Hyperglycaemia can induce excessive formation of reactive oxygen (ROS), ultimately leading to β -cells apoptosis related to oxidative stress (Lenzen 2008). β cells are vulnerable to oxidative stress because of lack of antioxidant

Fig. 5 Effect of SSE on the expression of Bax and Bcl-2. Western blotting analysis result of Bax and Bcl-2. **A** Bands of Bax, Bcl-2 and β-actin; **B** optical density analysis of Bax and

Bcl-2. Each value is expressed as the mean ± SD (n = 3). Values with different letters are significantly different at $p < 0.05$, as analyzed by Duncan's multiple range test

enzymes, which weaken the ability of β cells to defend against oxidative stress (Diplock et al. 1998). In order to relieve the pathological damage of β cells, oxidative stress caused by a high glucose level should be relieved (Valko et al. 2007). Thus, this study examined whether SSE reduced oxidative stress and protected INS-1 β cells from apoptosis, induced by high glucose concentrations.

High glucose concentrations in β cells increase the ROS levels, due to the overproduction of electron donors by the tricarboxylic acid cycle, which, in turn, increases oxidative stress (Robertson 2004). Excessive cellular levels of ROS can interact with nearby cellular components such as proteins, DNA, lipids, and cellular organs (e.g., mitochondria), to result in wide-ranging damage (Tiedge et al. 1997). Oxidative

Fig. 6 Effect of SSE on the expression of cytochrome *c*, caspase-9, and caspase-3. Western blotting analysis result of Bax and Bcl-2. **A** Bands of cytochrome *c*, caspase-9, caspase-3 and β-actin; **B** optical density analysis of cytochrome *c*,

caspase-9, and caspase-3. Each value is expressed as the mean ± SD (n = 3). Values with different letters are significantly different at $p < 0.05$, as analyzed by Duncan's multiple range test

stress exposure to β-cells activated the increased p21 cyclin-dependent kinase inhibitor production, decreased insulin mRNA, ATP and calcium flux reductions in mitochondria and cytosol to cause apoptosis (Maechler et al. 1999). In this study, SSE inhibited the apoptosis of beta cells by inhibiting the production of ROS.

SSE contains polyphenolic compounds, such as plastoquinones and phlorotannins. Plastoquinones are p-benzo quinone derivatives in seaweed or green plants with powerful antioxidant activity (Liu et al. 2012; Shohei et al. 2008). Phlorotannins are polyphenol compounds present in seaweed, which contain phloroglucinol (1,3,5-trihydroxybenzene) as their monomeric component (Liu et al. 2012). These

Fig. 7 Identification of the type of cell death by Annexin-V staining. Cells in 6-well plates (2×10^4 cells/well) were pre-incubated with 5.5 or 30 mM glucose of 48 h, and then incubated with or without the indicated concentrations of SSE for 48 h. Each value is expressed as the mean $\pm$ SD ($n = 3$).

Annexin V-FITC: Annexin V-fluorescein isothiocyanate. **A** 5.5 mM glucose, **B** 30 mM glucose, **C** 30 mM glucose + 0.05 mg/mL SSE, and **D** 30 mM glucose + 0.10 mg/mL SSE

Fig. 8 Effects of SSE on insulin secretion in high glucose-treated INS-1 cells. Cells in 10-mm dishes (3×10^5 cells/dish) were pre-incubated with glucose (5.5 or 30 mM) for 48 h, and then incubated with or without the indicated concentrations of SSE for 48 h. Insulin secretion from INS-1 cells in response to glucose (5 and 25 mM) was measured. Each value is expressed as the mean $\pm$ SD ($n = 3$). Values sharing the same superscript are not significantly different with $p < 0.05$

polyphenolic compounds exhibit strong antioxidant activity, acting as hydrogen or electron donating agents, and are considered one of the most important classes of natural antioxidants (Ryu et al. 2009; Balasundram et al. 2006). Based on these studies, the decreased ROS generation might be due to the plastoquinones and phlorotannins present in the SSE.

Increased oxidative stress is a consequence of either enhanced ROS production or attenuated ROS scavenging capacity, resulting in cell damage that can be assessed by the measurement of lipid peroxides. Thus, measuring lipid peroxidation could be a good indicator of the impairment of INS-1 β cell metabolism (Pi et al. 2007; Chikezie et al. 2015). In particular, lipid peroxidation, oxidative stress products exposed to β cell, inhibited insulin secretion (Miwa et al. 2000). This study suggests that SSE protects insulin secretion ability in β cells by reducing the formation of lipid peroxides.

Oxidative stress activates apoptosis signal transduction pathways in many cell types (Robinson and Cobb 1997; Simon et al. 2000; Jian 2007). Exposure to high levels of glucose in beta cells rapidly increases the formation of $O_2^{\cdot-}$ and NO (Giacco and Brownlee 2010; Mates 2000). Cytotoxicity as a result of a substantial NO-formation is established to initiate apoptosis, characterized by upregulation of the tumor suppressor p53, changes in the expression of pro- and

anti-apoptotic Bcl-2 family members, cytochrome *c* relocation, activation of caspases (Bernhard 2003). Mitochondrial-induced apoptosis releases cytochrome *c* into the cytosol, which causes assembly of a caspase-activating complex, referred to as the ‘apoptosome’ (Kuwana and Newmeyer 2003; Desagher and Martinou 2000). Bax is a pro-apoptotic protein that can promote apoptosis by inducing mitochondrial membrane permeability, resulting in the release of apoptogenic factors from the mitochondria while Bcl-2 is an anti-apoptotic protein preserves the integrity of the mitochondrial outer membrane (Nickells 1999). Changes in the levels of these gene products can further stimulate apoptotic events, including mitochondrial changes that ultimately lead to the activation of a family of cysteine proteases called caspases. Among them, caspase-3 and caspase-9 are frequently activated death proteases that catalyze the specific cleavage of many key cellular proteins (Porter and Jänicke 1999). In this study, a high glucose level caused apoptosis, via activation of the mitochondrial apoptotic pathway in INS-1 β cells. However, SSE treatment markedly reduced Bax expression, increased Bcl-2 expression, and also significantly decreased the activation of cytochrome *c*, caspase-3 and caspase-9. Some studies have reported that plastoquinones and phlorotannins have anti-apoptotic effects by acting as regulators of the mitochondrial apoptotic pathway (Mouria et al. 2002; Zhang et al. 2010). The anti-apoptotic effect of SSE may be due to the plastoquinones and phlorotannins it contains. A recent study has indicated that various forms of cellular stress result in mitochondrial alterations and the release of cytochrome *c*. Cytochrome *c* activates caspase-9, leading to the activation of other caspases (Green and Reed 1998), but polyphenol compounds inhibit transcription factor activation, thus regulating target genes involved in cell survival and proliferation (Surh et al. 2001). This study indicated that SSE protected the INS-1 β cells from apoptosis induced by a high level of glucose, which might be attributed to increased expression of anti-apoptotic Bcl-2 and decreased expression of pro-apoptotic Bax, cytochrome *c*, caspase-9, and caspase-3.

Exposure to a high glucose level increased the rate of apoptosis in INS-1 β cells (Lecoeur et al. 1997), as determined by flow cytometry with annexin-V as an apoptotic marker. However, SSE treatment reduced the rate of apoptosis in the high glucose-pretreated

cells, suggesting that SSE protected the INS-1 β cells from apoptosis induced by high glucose levels. β cells maintain glucose homeostasis by secreting insulin when the level of glucose increases (Paolisso et al. 1990), but the onset of apoptosis, induced by a high level of glucose in the β cells, decreases insulin secretion (Robertson 2004). In this study, exposure of INS-1 β cells to a high level of glucose significantly decreased insulin secretion, but SSE treatment markedly increased insulin secretion in the cells, showing that SSE could protect the cells from the damage caused by high glucose levels and contribute to the recovery of insulin secretion.

In conclusion, exposure of INS-1 β cells to a high level of glucose caused apoptosis, by increasing the levels of ROS, lipid peroxides, and NO. As a result, insulin secretion from the cells decreased, but SSE treatment significantly decreased lipid peroxide, intracellular ROS, and NO levels, and increased cell viability in high glucose-pretreated INS-1 cells. Furthermore, insulin secretion increased. These results suggest that SSE could be useful as a functional food to protect pancreatic β cells against oxidative stress and apoptosis, induced by high glucose levels.

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References

- Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem* 99:191–203
- Bernhard B (2003) Nitric oxide: NO apoptosis or turning it ON? *Cell Death Differ* 10:864–869
- Chikezie PC, Ojiako OA, Ogbuji AC (2015) Oxidative stress in diabetes mellitus. *Int J Biol Chem* 9:92–109
- Choi BW, Ryu G, Park SH et al (2007) Anticholinesterase activity of plastoquinones from *Sargassum sagamianum*: lead compounds for Alzheimer's disease therapy. *Phytother Res* 21:423–426
- Choi JS, Ha YM, Joo CU, Cho KK, Kim SJ, Choi IS (2012) Inhibition of oral pathogens and collagenase activity by seaweed extracts. *J Environ Biol* 33:115–121
- Desagher S, Martinou JC (2000) Mitochondria as the central control point of apoptosis. *Trends Cell Biol* 10:369–377
- Diplock AT, Charuleux JL, Crozier-Willi G et al (1998) Functional food science and defence against reactive oxidative species. *Br J Nutr* 80:77–112
- Donath MY, Halban PA (2004) Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia* 47:581–589
- Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. *Circ Res* 107:1058–1070
- Green DR, Reed JC (1998) Mitochondria and apoptosis. *Science* 281:1309–1312
- Horie S, Tsutsumi S, Takada Y, Kimura J (2008) Antibacterial quinone metabolites from the brown alga, *sargassum sagamianum*. *Bull Chem Soc Jpn* 81:1125–1130
- Jian Z (2007) Interplay among nitric oxide and reactive oxygen species a complex network determining Cell survival or death. *Plant Signal Behav* 2:544–547
- Kaneto H, Kajimoto Y, Miyagawa JI et al (1999) Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 48:2398–2406
- Kim MJ, Kim KBWR, Lee CJ, Kwak JH, Kim DH, Sunwoo C, Jung SA, Kang JY, Kim HJ, Choi JS (2011) Korean J Food Sci Technol 43:723–728
- Kim MJ, Jeong DH, Ahn DH (2013) Anti-inflammatory activity of ethanolic extract of *Sargassum sagamianum* in RAW 264.7 cells. *Food Sci Biotechnol* 22:1113–1120
- Kuwana T, Newmeyer DD (2003) Bcl-2-family proteins and the role of mitochondria in apoptosis. *Curr Opin Cell Biol* 15:691–699
- Lecoeur H, Ledru E, Prévost MC, Gougeon ML (1997) Strategies for phenotyping apoptotic peripheral human lymphocytes comparing ISNT, annexin-V and 7-AAD cytofluorometric staining methods. *J Immunol Methods* 209:111–123
- Lee JS, Han JS (2018) *Sargassum sagamianum* extract alleviates postprandial hyperglycemia in diabetic mice. *Prev Nutr Food Sci* 23:122–126
- Lenzen S (2008) Oxidative stress: the vulnerable beta-cell. *Biochem Soc Trans* 36:343–347
- Liu L, Heinrich M, Myers S, Dworjany SA (2012) Towards a better understanding of medicinal uses of the brown seaweed *sargassum* in traditional chinese medicine: a phytochemical and pharmacological review. *J Ethnopharmacol* 142:591–619
- Maechler P, Jornot L, Wollheim CB (1999) Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. *J Biol Chem* 274:27905–27913
- Mates JM (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 153:83–104
- Miwa I, Ichimura N, Sugiura M, Hamada Y, Taniguchi S (2000) Inhibition of glucose-induced insulin secretion by 4-hydroxy-2-nonenal and other lipid peroxidation products. *Endocrinology* 141:2767–2772
- Mouria M, Gukovskaya AS, Jung Y et al (2002) Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis. *Int J Cancer* 98:761–769
- Nickells RW (1999) Apoptosis of retinal ganglion cells in glaucoma: an update of the molecular pathways involved in cell death. *Surv Ophthalmol* 43:151–161
- Paneni F, Beckman HA, Creager MA, Cosentino F (2013) Diabetes and vascular disease: pathophysiology, clinical

- consequences, and medical therapy: part I. *Eur Heart J* 34:2436–2443
- Paolisso G, Scheen A, d'Onofrio F, Lefebvre P (1990) Magnesium and glucose homeostasis. *Diabetologia* 33:511–514
- Pi J, Bai Y, Zhang Q, Wong V, Floering LM, Daniel K et al (2007) Reactive oxygen species as a signal in glucose-stimulated insulin secretion. *Diabetes* 56:1783–1791
- Porter AG, Jänicke RU (1999) Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 6:99
- Robertson RP (2004) Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem* 279:42351–42354
- Robertson R, Zhou H, Zhang T, Harmon JS (2007) Chronic oxidative stress as a mechanism for glucose toxicity of the beta cell in type 2 diabetes. *Cell Biochem Biophys* 48:139–146
- Robinson MJ, Cobb MH (1997) Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* 9:180–186
- Ryu B, Li Y, Qian ZJ, Kim MM, Kim SK (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: implication for chronic articular disease. *Chem Biol Interact* 179:192–201
- Shohei H, Shinsuke T, Yuuki T, Junji K (2008) Antibacterial quinone metabolites from the brown alga, *Sargassum sagamianum*. *Bull Chem Soc Jpn* 81:1125–1130
- Simon HU, Haj-Yehia A, Levi-Schaffer F (2000) Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis* 5:415–418
- Surh YJ, Chun KS, Cha HH et al (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF- κ B activation. *Mutat Res Fundam Mol Mech Mutagen* 480:243–268
- Talchai C, Xuan S, Lin HV, Sussel L, Accili D (2012) Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* 150:1223–1234
- Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 46:1733–1742
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39:44–84
- Zhang R, Lee IK, Kang KA et al (2010) Cytoprotective effects of triphlorethol-A against formaldehyde-induced oxidative damage and apoptosis: role of mitochondria-mediated caspase-dependent pathway. *J Toxicol Environ Health A* 73:1477–1489

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