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Ferulic Acid Protects INS-1 Pancreatic β Cells Against High Glucose-Induced Apoptosis

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Diabetes mellitus (DM) is one of the main global health problems. Chronic exposure to hyperglycemia can lead to cellular dysfunction that may become irreversible over time, a process that is termed glucose toxicity. Our perspective about glucose toxicity as it pertains to the pancreatic β-cell is that the characteristic decreases in insulin secretion are caused by regulated apoptotic gene expression. In this study, we examined whether ferulic acid protects INS-1 pancreatic cells against high glucose-induced apoptosis. High glucose concentration (30 mM) induced glucotoxicity and death of INS-1 pancreatic β cells. However, treatment with 1, 5, 10, or 20 μM ferulic acid increased the cell viability in a concentration-dependent manner. Treatment with ferulic acid dose-dependently decreased the intracellular levels of reactive oxygen species, thiobarbituric acid reactive substances, and nitric oxide in INS-1 pancreatic β cells pretreated with high glucose. These effects influence the apoptotic pathway, increasing the expression of the anti-apoptotic protein Bcl-2 and reducing the levels of pro-apoptotic proteins, including Bax, cytochrome C, and caspase 9. Annexin V/propidium iodide staining indicated that ferulic acid significantly reduced high glucose-induced apoptosis. These results demonstrate that ferulic acid is a potential therapeutic agent to protect INS-1 pancreatic β cells against high glucose-induced apoptosis.

Key words : Apoptosis, ferulic acid, high glucose, oxidative stress, pancreatic β cells

Introduction

Type 2 diabetes is characterized by high blood-glucose levels attributed to insulin resistance and defective 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 [5, 9]. Insulin is a hormone secreted by pancreatic beta cells that maintains blood glucose homeostasis [1]. Persistent hyperglycemia has toxic effects on the cellular function and structure and increases the apoptosis in type 2 diabetes. This results in reduced insulin secretion, thereby exacerbating diabetes [3, 5]. Hence, to prevent pancreatic β-cell dysfunction and apoptosis, it is important to reduce the oxidative stress and apoptosis caused by hyperglycemia.

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Hyperglycemia produces high levels of ROS in the mitochondria, which induce oxidative stress. Antioxidant enzyme systems protect cells from ROS; however, excessive accumulation of oxidants can lead to an imbalance between oxidants and antioxidants [37]. Oxidative stress causes severe damage and can lead to apoptosis [12]. Oxidative stress-sensitive β cells are easily damaged by high glucose levels, causing apoptosis and further reducing insulin secretion [28]. Thus, mitigating pathological apoptosis of β cells impaired by elevated glucose levels is a necessity. Furthermore, reducing glucotoxicity attributed to high glucose levels is also imperative.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid), a hydroxycinnamic acid derived from various seeds, nuts, grains, and vegetables, exists in its free form [11]. It exhibits various pharmacological effects, including anti-inflammatory, antimicrobial, and anticancer activities [7]. Particularly, ferulic acid is considered a superior antioxidant [34]. However, the effects of ferulic acid on pancreatic β-cell dysfunction and survival under high glucose concentrations have not been examined. Therefore, this study investigated whether ferulic acid could protect pancreatic β cells from glucotoxicity attributed to high glucose levels.

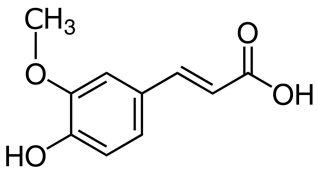

Fig. 1. Chemical structure of ferulic acid.

Materials and Methods

Materials

Ferulic acid (Fig. 1) was obtained from Sigma-Aldrich (St. Louis, MO, USA). RPMI 1640 medium was acquired from the Welgene Company (Gyeongsan, Gyeongsangbuk-do, Korea), and other chemicals, including fetal bovine serum (FBS), streptomycin, and penicillin, were acquired from Sigma-Aldrich. The insulin enzyme-linked immunosorbent assay (ELISA) kit was acquired from LINCO Research, Inc. (St. Charles, MO, USA).

Cell culture

INS-1 pancreatic β cells were cultured in RPMI 1640 medium (Welgene), supplemented with 10% FBS, 100 units/ml streptomycin, 100 μg/ml penicillin, and 50 μM beta-mercaptoethanol, at 37°C in a humidified atmosphere containing 5% CO_2.

Cell viability assay

Cell viability was confirmed using a colorimetric MTT assay. The cells (2×10^4) were pretreated with glucose (5.5 or 30 mM) in a 96-well plate for 48 hr, followed by treatment with 0, 1, 5, 10, or 20 μM ferulic acid for 48 hr. This sample treatment was followed for all subsequent experiments. The cells were treated with MTT solution (100 μl) in 96-well plates, and after 4 hr at 37°C, the formazan crystals formed in viable cells were melted with 100 μl of dimethyl sulfoxide. The absorbance of each well was measured at 540 nm using a microplate reader.

Assay to examine intracellular reactive oxygen species (ROS)

Intracellular ROS levels were measured using a dichlorofluorescein assay. After sample treatment, cells were treated with 5 μM of DCF-DA solution and incubated for 30 min. In the presence of radicals, DCF-DA transformed into a fluorescent product that was maintained within the cells. The fluorescence was measured at 530 nm using a fluorescence plate reader (LS-3B; PerkinElmer, Waltham, MA, USA).

Assay to examine lipid peroxidation

Lipid peroxidation was confirmed by thiobarbituric acid reactive substance (TBARS) assay. After sample treatment, the culture supernatants were collected, and 400 μl of TBARS solution was added. The mixture was then boiled at 95°C for 20 min, and the absorbance was measured at 532 nm. TBARS values were calculated as nmol malondialdehyde using a 1,1,3,3-tetraethoxypropane serial dilution curve.

Nitric oxide (NO) assay

NO levels in cell culture supernatants were measured using the Griess reaction. The cell culture supernatant (50 μl) was mixed with an equal volume of Griess reagent [0.1% N-(1-naphthyl)-ethylenediamine and 1% sulfanilamide in 5% phosphoric acid], and the mixture was incubated at room temperature for 10 min. Absorbance was measured at 550 nm using a microplate absorbance reader. A series of known concentrations of sodium nitrite was used as the standards.

Glucose-stimulated insulin secretion (GSIS)

INS-1 cells (2×10^4 cells/well) were pre-incubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr. Thereafter, the cells were washed, and the cell-culture medium was replaced with fresh medium and incubated for 5 hr. Subsequently, the cells were incubated with ferulic acid (0, 1, 5, 10, or 20 μM) for 48 hr and stimulated with Krebs－Ringer buffer, containing 5 or 25 mM glucose, for 1 hr. Cell media were collected to measure insulin secretion. Insulin secretion was analyzed using an insulin ELISA kit (LINCO Research Inc.).

Western blot analysis

The cells were lysed in a cold lysis buffer containing 250 mM NaCl, 25 mM Tris-HCl, 1%(v/v) NP-40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and a protease inhibitor cocktail. Protein concentrations were determined using with a BCATM protein assay kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (20 μg/lane) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. Membranes were probed with secondary antibodies. Detection was performed using an Enhanced chemiluminescence (ECL) western blotting detection kit (Bio-Rad). Multi Gauge v3.1 was used to quantify relative protein expression (FujiFilm, Tokyo, Japan).

Flow cytometric assessment of apoptosis

Cells were treated with 5.5 mM glucose, 30 mM glucose, or ferulic acid to quantify apoptosis using annexin V/propidium iodide (PI) staining. The cell suspension (100 μl) was treated with 5 μl annexin-V and PI. After 15 min in the dark bath, this mixture was subjoined with 400 μl binding buffer, which was then analyzed by flow cytometry (Becton Dickinson, San Jose, CA, USA).

Statistical analysis

All experiments were conducted in triplicates. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using the SAS software (SAS Institute, Inc., Cary, NC, USA). The treatment groups were compared using one-way analysis of variance (ANOVA), followed by a post hoc Duncan's multiple-range test. Statistical significance threshold was set at $p<0.05$.

Results

Effect of ferulic acid on cell viability

The effect of ferulic acid on the viability of INS-1 pancreatic β cells was investigated using an MTT assay (Fig. 2). In β cells treated with a high glucose concentration of 30 mM, the viability was significantly lower (53.38%) compared to that in cells treated with a normal glucose concentration of 5.5 mM. However, treatment with 1, 5, 10, or 20 μM of ferulic acid significantly increased the viability rate to 55.73, 77.71, 81.30, and 89.06%, respectively.

Effect of ferulic acid on intracellular reactive oxygen species (ROS)

In pancreatic β cells treated with 30 mM high glucose, the level of intracellular ROS was significantly increased by 208.50% compared with cells treated with 5.5 mM normal glucose (Fig. 3). However, following treatment with ferulic acid, ROS levels decreased in a dose-dependent manner. Treatment with 1, 5, 10 and 20 μM ferulic acid significantly reduced high glucose-induced ROS production (201.87, 163.30, 148.60 and 125.24%, respectively). Thus, ferulic acid significantly reduced intracellular ROS excessively produced in response to high glucose levels in pancreatic β cells.

Effect of ferulic acid on the production of TBARS

The effect of ferulic acid on lipid peroxidation was investigated by measuring the lipid peroxidation product, TBARS (Fig. 4). The TBARS levels in cells treated with normal and high glucose concentrations were 0.14 and 0.29 nmol of MDA, respectively. Treatment with 1, 5, 10, or 20 μM of ferulic acid significantly lowered high-glucose-induced TBARS production to 0.28, 0.25, 0.22, and 0.18 nmol MDA, respectively.

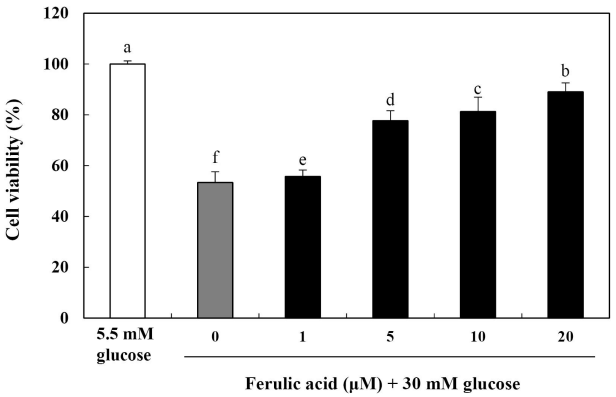

Fig. 2. Effect of ferulic acid on the viability of high glucose-treated INS-1 cells. INS-1 cells (2×10^4 cells/well) are preincubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr and then incubated with ferulic acid (0, 1, 5, 10, or 20 μM) for 48 hr. Here, 5.5 mM and 30 mM of glucose represents normal and high glucose levels, respectively. Each value is expressed as the mean ± SD (n=3). $^{a\text{-}f}$Values with different letters indicate significant differences with $p<0.05$, as analyzed using Duncan's multiple-range test.

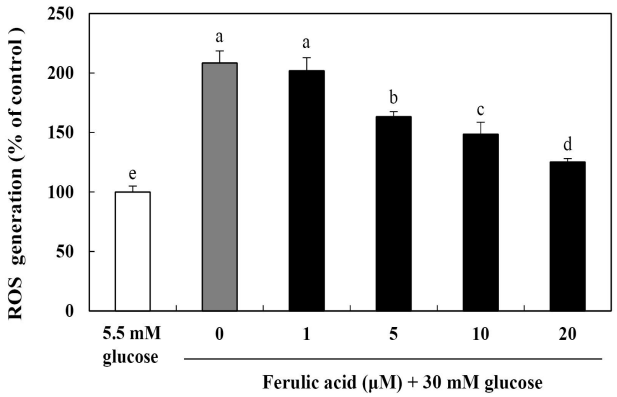

Fig. 3. Effect of ferulic acid on the intracellular levels of ROS in high glucose-treated INS-1 cells. INS-1 cells (2×10^4 cells/well) are preincubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr and then incubated with ferulic acid (0, 1, 5, 10, or 20 μM) for 48 hr. Here, 5.5 mM and 30 mM of glucose represents normal and high glucose levels, respectively. Each value is expressed as the mean ± SD (n=3). $^{a\text{-}e}$Values with different letters indicate significant differences with $p<0.05$, as analyzed using Duncan's multiple-range test.

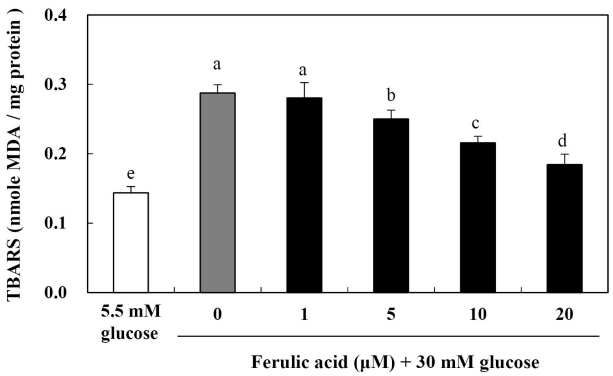

Fig. 4. Effect of ferulic acid on the intracellular levels of TBARS in high glucose-treated INS-1 cells. INS-1 cells (2×10^4 cells/well) are preincubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr and then incubated with ferulic acid (0, 1, 5, 10, or 20 μM) for 48 hr. Here, 5.5 mM and 30 mM of glucose represents normal and high glucose levels, respectively. Each value is expressed as the mean ± SD (n=3). $^{a-e}$Values with different letters indicate significant differences with $p<0.05$, as analyzed by Duncan's multiple-range test.

Effect of ferulic acid on NO levels

Treatment of INS-1 pancreatic β cells with 30 mM glucose significantly increased NO levels to 264.34% compared with control cells treated with 5.5 mM glucose (Fig. 5). However, cells pretreated with ferulic acid before exposure to 30 mM glucose displayed decreased levels of NO in an ferulic acid dose-dependent manner. The NO production induced by the high glucose concentration in the 1, 5, 10 and 20 μM ferulic acid pretreated cells was 253.27, 215.60, 210.89 and 175.73 %, respectively. Thus, ferulic acid progressively inhibited NO production caused by high glucose.

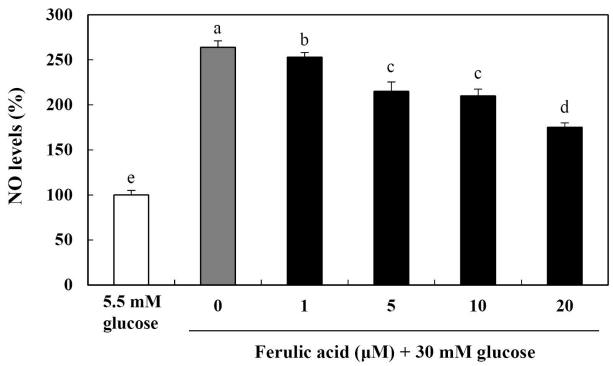

Fig. 5. Effect of ferulic acid on the intracellular levels of NO in high glucose-treated INS-1 cells. INS-1 cells (2×10^4 cells/well) are preincubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr and then incubated with ferulic acid (0, 1, 5, 10, or 20 μM) for 48 hr. Here, 5.5 mM and 30 mM of glucose represents normal and high glucose levels, respectively. Each value is expressed as the mean ± SD (n=3). $^{a-e}$Values with different letters indicate significant differences with $p<0.05$, as analyzed by Duncan's multiple-range test.

Effect of ferulic acid on the expression of apoptosis-related proteins

Western blot analysis was performed to determine if ferulic acid alters the expression of genes involved in apoptosis induced by high glucose levels. The levels of Bax, Bcl-2, cytochrome c, caspase 9, and caspase 3 were measured. As shown in Fig. 6, the expression levels of pro-apoptotic Bax, cytochrome c, caspase 9, and caspase 3 were significantly increased in INS-1 pancreatic β cells treated with high glucose. However, the expression levels of anti-apoptotic Bcl-2 were significantly low in cells treated with high glucose. Treatment with 5 or 20 μM ferulic acid significantly decreased the levels of Bax, cytochrome c, caspase 9, and caspase 3 and significantly increased the levels of Bcl-2. The results showed that ferulic acid treatment decreased the expression of pro-apoptotic proteins and increased the expression of anti-apoptotic proteins in the β cells damaged by high glucose.

Cell apoptosis rate measured by annexin-V/PI staining

After treatment with ferulic acid, the apoptosis rate was assessed in the β cells by flow cytometry using annexin-V/PI staining. Viable cells are shown in the lower left quadrant, and cells in the early and late stages of apoptosis are shown at the bottom and top of the right quadrant, respectively (Fig. 7). Under a high-glucose condition (30 mM), apoptosis levels were significantly increased compared to those under a normal-glucose condition (5.5 mM). However, the addition of 5 or 20 μM ferulic acid reduced the rate of death in cells treated with 30 mM glucose. When the cells were treated with 30 mM glucose alone, the rates of early and late apoptosis significantly increased. However, the rate of early and late cell death was significantly decreased following treatment with 20 μM ferulic acid. The results indicated that ferulic acid protected the β cells from apoptosis caused by high glucose levels.

Effect of ferulic acid on insulin secretion

Fig. 8 shows that the insulin secretion in INS-1 pancreatic β cells was significantly decreased in response to high-glucose (30 mM) treatment compared to that observed with normal-glucose (5.5 mM) treatment. After inducing glucose tox-

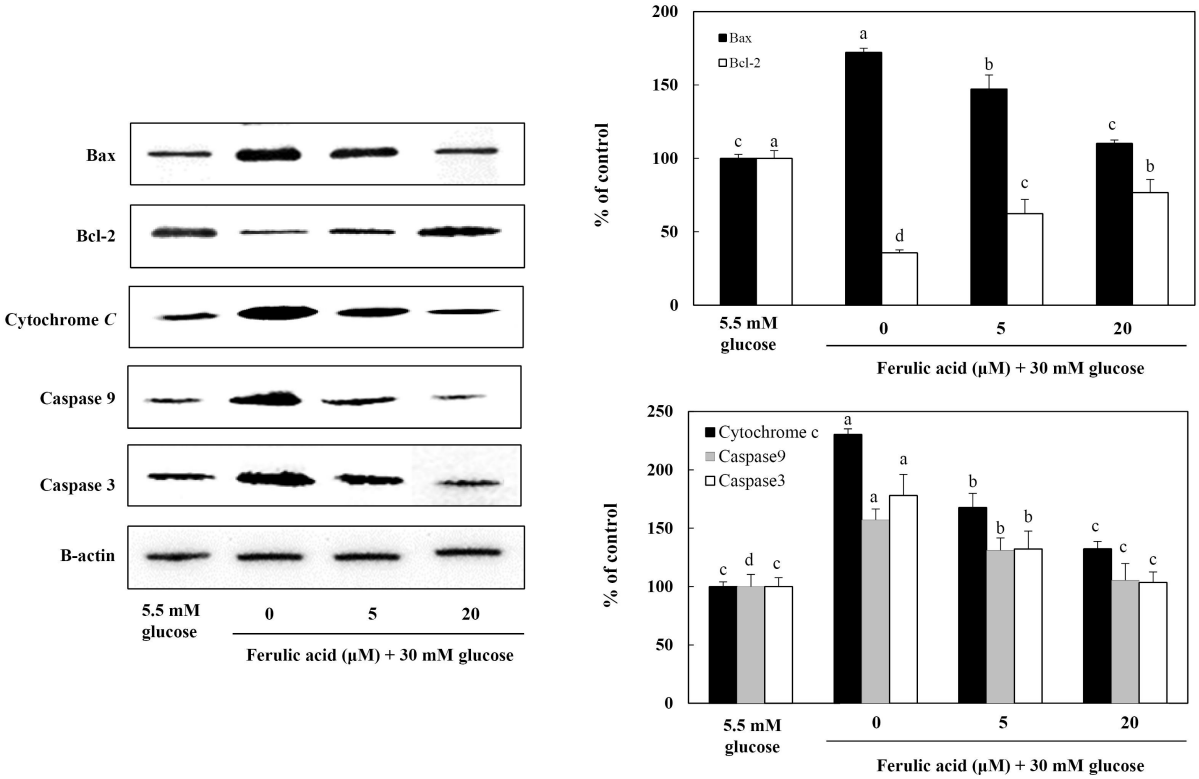

Fig. 6. Effect of ferulic acid on the expression of Bax, Bcl-2, cytochrome C, caspase 9, and caspase 3 proteins in high glucose-treated INS-1 cells. INS-1 cells are preincubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr and then incubated with ferulic acid (0, 5, or 20 μM) for 48 hr. Here, 5.5 mM and 30 mM of glucose represents normal and high glucose levels, respectively. Equal amounts of cell lysates were electrophoresed and analyzed for Bax, Bcl-2, cytochrome C, caspase 9, and caspase 3 proteins using western blotting. Actin was used as an internal control. (A) Expression of Bax, Bcl-2, cytochrome C, caspase 9, and caspase 3 proteins (B) Expression of Bax, Bcl-2, cytochrome C, caspase 9, and caspase 3 proteins. Each value is expressed as the mean ± SD (n=3). [a-d]Values with different letters indicate significant differences with $p<0.05$, as analyzed by Duncan's multiple-range test.

icity by treatment with 30 mM glucose and washing, the insulin secretion ability was analyzed by treatment with 5 or 25 mM glucose. Treatment with ferulic acid restored the 30 mM glucose-induced impairment of insulin secretion with 5 or 25 mM glucose. Treatment of INS-1 pancreatic β cells with 1, 5, 10, and 20 μM ferulic acid increased insulin secretion by 6.48, 11.08, 15.98, and 20.41 ng/hr, respectively in cells incubated with 5 mM glucose ($p<0.05$). In addition, ferulic acid treatment at 1, 5, 10, and 20 μM concentrations increased insulin secretion by 13.46, 17.71, 19.54, and 24.30 ng/hr in cells incubated with 25 mM glucose ($p<0.05$). The results indicated that ferulic acid alleviated the insulin secretion reduction in the β cells, induced by high-glucose concentrations.

Discussion

Pancreatic β cells secrete insulin, an important hormone for maintaining blood glucose homeostasis. Blood-glucose levels are normally maintained by the balance between insulin secretion and action. However, dysfunction of the β cells is a critical factor in the pathogenesis of diabetes as it reduces insulin secretion. In pancreatic β cells, oxidative phosphorylation of glucose produces ROS. The effects of ROS are mitigated by antioxidant enzymes, such as superoxide dismutase and catalase. β cells produce low levels of these enzymes and glutathione peroxidase (a redox-regulating enzyme) and are sensitive to oxidative stress [16, 27]. Under pathological conditions of hyperglycemia, high glucose levels can disrupt glycolysis, and glucose can be metabolized via pathways that produce excessive amounts of ROS in the mitochondria. Excessive ROS production leads to oxidative stress, eventually damaging β cells that produce relatively low levels of antioxidant enzymes, such as catalase and glutathione peroxidase [2]. Reducing oxidative stress attributed to hyperglycemia is important to restore β-cell damage in type

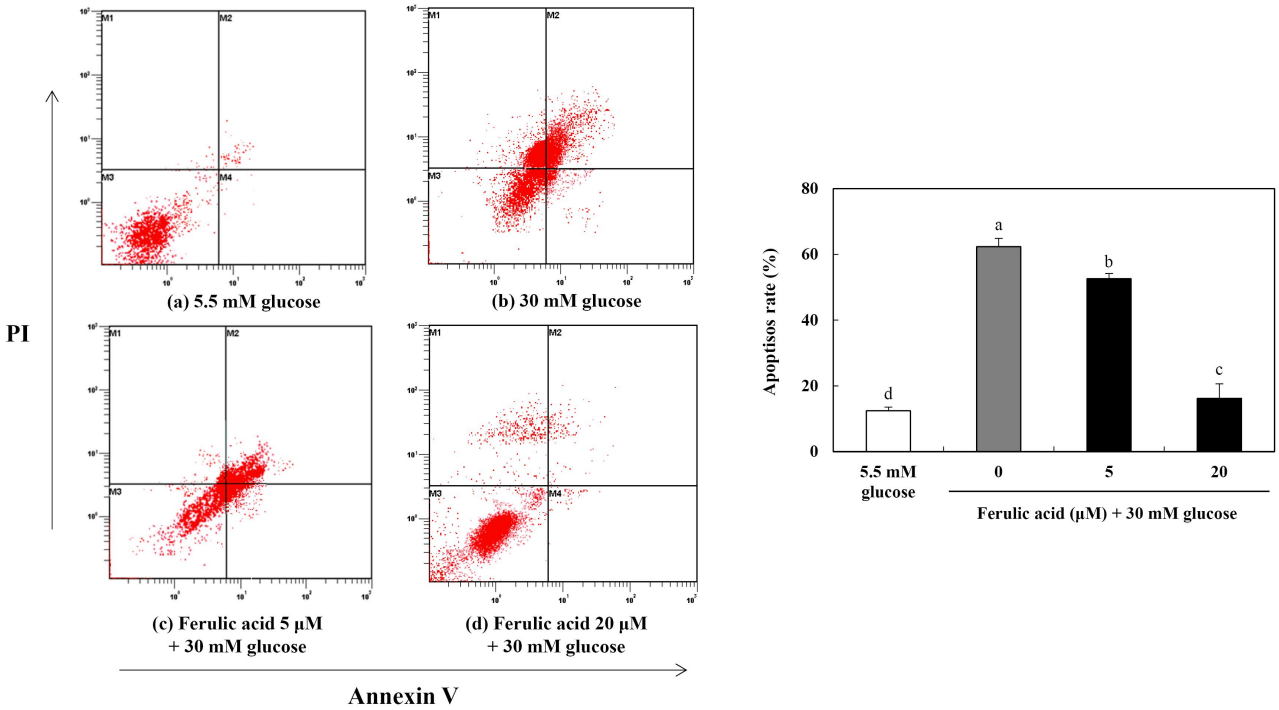

Fig. 7. Identification of the apoptotic stage of INS-1 cells in response to different treatments using annexin-V/ PI staining. (A) The status of apoptotic cell death is determined by counting INS-1 cells stained with annexin V-FITC/PI using a flow cytometer. Cells are preincubated with glucose and then incubated in the presence or absence of ferulic acid (0, 5, or 20 μM). The right lower and upper quadrants show the numbers of early and late apoptotic cells, respectively. (B) Mean rates of apoptosis. $^{a-d}$Values with different letters indicate significant differences with $p<0.05$, as analyzed by Duncan's multiple-range test.

2 diabetes.

Ferulic acid is a hydroxycinnamic acid with low toxicity and numerous physiological functions, including anti-inflammatory, antimicrobial, anticancer, antidiabetic, and immunostimulatory effects [17, 34]. Among these, antioxidant activity has been the most documented [26]. Therefore, we investigated whether ferulic acid protected pancreatic cells from oxidative stress and cell damage caused by high glucose levels. The MTT assay was performed to assess the viability of β cells. High glucose concentrations of 30 mM significantly reduced the viability of β cells. However, ferulic acid significantly restored the cell viability. These results indicated that ferulic acid protected β cells against cytotoxicity attributed to high glucose.

High glucose levels accelerate metabolic reactions leading to excessive ROS production. Excessive ROS levels affect the function and survival of cells by activating signaling pathways that are sensitive to cellular stress [30]. Activation of apoptosis by ROS leads to diabetes and worsens diabetic conditions. Hence, the inhibition of excessive ROS production induced by high glucose concentrations may contribute to alleviating β-cell impairment, thereby preventing the progression of diabetes [19, 29]. When pancreatic β cells were treated with 30 mM of glucose, intracellular ROS levels were significantly increased. However, ferulic acid treatment inhibited this production, resulting in significantly lower ROS levels. This result indicates that ferulic acid may prevent pancreatic cell impairment at high glucose concentrations by lowering ROS production. Ferulic acid belongs to the phenolic acid group and has several functional groups (one hydroxyl and one methoxy group) in its structure [15]. The number and position of hydroxyl groups in phenolic acids are directly related to their free-radical-scavenging ability [25]. When the number of phenolic hydroxyl groups on the benzene ring is less than four, the antioxidant activity of the phenolic acids is proportional to the number of phenolic hydroxyl groups [8]. Hence, the scavenging effects of ferulic acid on ROS may be attributed to the presence of hydroxyl groups in its structure.

Increased ROS levels cause cell-membrane damage via the formation of lipid peroxides and lipid peroxide radicals. Hence, lipid peroxidation may serve as a valuable indicator

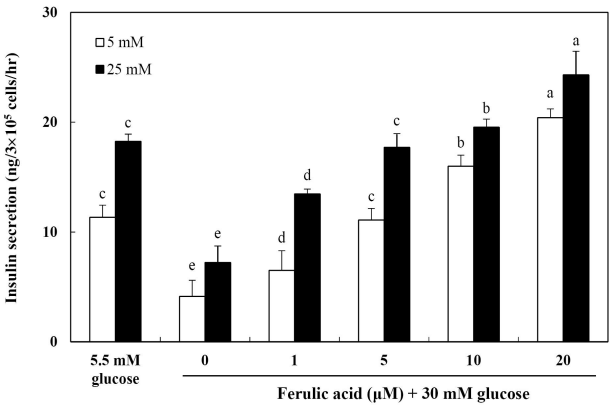

Fig. 8. Effect of ferulic acid on the insulin secretion in high-glucose-treated INS-1 cells. INS-1 cells (2×10^4 cells/well) are pre-incubated in 96-well plates with 5.5 or 30 mM of glucose for 48 hr. Thereafter, the cells are washed, and the cell culture medium is replaced with fresh medium and further incubated for 5 hr. Subsequently, the cells are incubated with ferulic acid (0, 1, 5, 10, or 20 μM) for 48 hr and stimulated with Krebs-Ringer buffer containing 5 or 25 mM glucose for 60 min. The concentrations of 5.5 mM and 30 mM represent normal and high glucose concentrations, respectively. Each value is expressed as the mean ± SD (n= 3). [a-e]Values with different letters indicate significant differences with $p<0.05$, as analyzed by Duncan's multiple-range test.

of impairment in INS-1 pancreatic β cells [6, 21]. Exposure of INS-1 pancreatic β cells to high glucose levels increased the generation of lipid peroxides; however, ferulic acid treatment significantly inhibited lipid-peroxide generation. This result suggests that the inhibition of lipid-peroxide formation may be one of the protective mechanisms involved in the ferulic acid-mediated reduction of high glucose-induced cytotoxicity in INS-1 pancreatic β cells. Lipid peroxidation is mediated by ROS and can induce β-cell dysfunction and reduce insulin secretion [4]. Therefore, the production of TBARS should be prevented, and β-cell malfunction due to oxidative stress should be reduced. Flavonoids can inhibit TBARS production due to their antioxidant activity [23].

Oxidative stress activates apoptotic signal-transduction pathways in many cell types [36]. It also promotes apoptosis by participating in the processes related to the initiation of apoptotic signal transduction. Mitochondria are known to induce apoptosis by releasing cytochrome c into the cytosol, which leads to the assembly of a caspase-activating complex referred to as the apoptosome [10]. Bax is a pro-apoptotic protein that promotes apoptosis, resulting in the release of apoptotic factors from mitochondria. In contrast, Bcl-2 is an anti-apoptotic protein that preserves the integrity of the outer mitochondrial membrane [24]. Changes in the expression of these genes can further stimulate apoptotic events, including changes in the mitochondria, which ultimately lead to the activation of a family of cysteine proteases called caspases [22]. Among these, caspase 3 and caspase 9 are frequently activated death proteases that catalyze the specific cleavage of many key cellular proteins [20]. In this study, high glucose levels induced the release of apoptogenic factors via activation of the mitochondrial apoptotic pathway in INS-1 pancreatic β cells. However, ferulic acid treatment markedly reduced Bax expression, increased Bcl-2 expression, and significantly decreased the levels of cytochrome c, caspase 3, and caspase 9.

In some studies, the hydroxyl groups of compounds isolated from plants have exhibited anti-apoptotic effects and are involved in the regulation of mitochondrial apoptotic pathways [14, 33]. The anti-apoptotic effect of the hydroxyl group is related to the inhibition of intracellular ROS and lipid peroxidation. Particularly, the hydroxyl group in food-derived polyphenols helps scavenge free radicals and is considered to contribute to their antioxidant activity [18, 35]. Polyphenolic hydroxyl groups release active hydrogen atoms to block lipid autoxidation and regulate mitochondrial membranes to maintain the levels of apoptotic and anti-apoptotic proteins [32, 35]. Ferulic acid has one hydroxyl group at the C-4 position of the benzene ring. Hence, we hypothesized that the hydroxyl group in ferulic acid might contribute to its anti-apoptotic effect in INS-1 pancreatic β cells. This study suggested that ferulic acid protects INS-1 pancreatic β cells from apoptosis induced by high glucose levels. This effect was confirmed by the increased anti-apoptotic Bcl-2 expression and decreased expression of pro-apoptotic Bax, cytochrome c, caspase 9, and caspase 3.

Flow cytometry was performed to examine the rate of cell death using annexin V as a marker [31]. After treatment with high glucose, cells in the pancreas showed an increased rate of cell death; however, ferulic acid markedly decreased the number of apoptotic cells. This result indicates that ferulic acid can protect the β cells from hyperglycemia-mediated cell death. Hyperglycemia-induced damage to pancreatic cells can lead to insulin-secretion deficiency [13]. In this study, insulin secretion markedly decreased in cells treated with high glucose at a concentration of 30 mM. However, treatment with ferulic acid significantly increased insulin secretion. These results imply that ferulic acid could protect pancreatic β cells and restore their insulin-secretion capacity.

In conclusion, treatment with high glucose (30 mM) con-

centrations increased the ROS, TBARS, and NO levels. As a result, apoptosis increased and insulin secretion decreased. However, ferulic acid treatment protected the β cells of pancreas from the effects of high glucose concentration by lowering cell death, which significantly restored the level of insulin secretion. Our results demonstrated the efficacy of ferulic acid in protecting cells from glucotoxicity and preventing the progression of diabetes. Hence, ferulic acid shows promise as a pharmaceutical agent that protects cells from hyperglycemia-induced apoptosis.

Acknowledgement

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : INS-1 췌장 베타 세포에서 ferulic acid의 당독성 개선 효과

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제 2형 당뇨병에서 나타나는 인슐린 분비 감소는 베타세포의 자가사멸에 의한 베타세포질량의 급격한 감소로 인한 것으로 보고되고 있으며, 베타세포의 자가사멸을 촉진하는 요인으로 고혈당에 의한 당독성 및 활성산소종들의 증강에 의한 산화스트레스 등이다. Ferulic acid는 항산화, 항염, 항암 등 다양한 생리활성을 나타내며, 본 연구에서는 고혈당으로 유도된 세포 당독성 개선 효과와 그 기전을 INS-1 췌장 베타세포에서 규명하고자 하였다. Ferulic acid는 고농도 포도당 처리된 INS-1 췌장 베타 세포에서 세포 생존율을 증가시키고, 지질과산화물, 세포 내 ROS 및 NO 수준을 감소시켰다. 세포사멸 관련 인자의 유전자 발현 결과 pro-세포자가사멸 인자인 bax, cytochrome c, caspase-3 및 caspase-9의 단백질 발현을 유의적으로 감소시켰고, anti-세포자가사멸 인자인 bcl-2 발현을 증가시켰다. Ferulic acid는 annexin V/I propidium iodide 분석을 통하여 고농도 포도당으로 유도된 세포 사멸을 감소시키고, INS-1 췌장 베타세포에서의 인슐린 분비능을 증가시키는 것으로 사료된다. 따라서ferulic acid는 고농도 포도당으로 손상된 INS-1 췌장 베타세포의 보호효과를 나타낸다.