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Therapeutic potential of genistein in ovariectomy-induced pancreatic injury in diabetic rats: The regulation of MAPK pathway and apoptosis

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ABSTRACT

Objective(s): Genistein, as a phytoestrogen found in legumes, has several biological activities in general and anti-diabetic activity particularly. In this study, we investigated the effect of genistein on proteins involved in β-cell proliferation, survival and apoptosis to further reveal its anti-diabetic potential in the ovariectomized diabetic rat.

Materials and Methods: We used three-month-old female Wistar rats that either underwent ovariectomy (OVX) or received a sham surgery (Sham). In a subsequent series of experiments, OVX rats received high-fat diet and low dose STZ to induce diabetes (OVX.D) and genistein treatment (OVX.D.G). Western blot analysis was used for the assessment of phosphorylation of ERK1/2 and AKT and expression of Bcl-2 and caspase-3 in pancreas tissue. Hematoxylin-Eosin (H&E) staining was used for histopathological assessment.

Results: Genistein induced AKT and ERK1/2 phosphorylation protein expression of Bcl-2 in the pancreas. In addition, genistein suppressed protein level of caspase-3. Administration of genistein significantly improved hyperglycemia in ovariectomized diabetic rat, concomitant with improved islet β-cell morphology and mass.

Conclusion: These findings suggest that the beneficial antidiabetic effect of genistein partially mediated by directly modulating pancreatic β-cell function via activation of the AKT, ERK1/2, and Bcl-2, as cell survival and anti-apoptotic factors, and decreasing of proapoptotic caspase-3.

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Introduction

The postmenopausal years are accompanied with an increase in glucose levels, and with rising visceral adiposity (1). Though numerous studies into the impact of menopause on diabetes have been undertaken, the relationship remains unclear (2, 3). The relationship between diabetes and estrogens is also unclear, although estrogen replacement therapy has been suggested to decrease diabetes risk in postmenopausal women (4). The noticeable decay in endogenous estrogen levels after menopause, causing raised relative androgenicity and changes in body composition, is indicated to affect pancreatic β-cell function, hepatic glucose output, and insulin-induced glucose transport (5).

Bilateral ovariectomy abruptly decreases estrogen production, that is followed by post glucose-induced hyperinsulinemia, suggesting insulin resistance (6). In rats, bilateral ovariectomy reduced insulin-mediated glucose uptake through an impaired insulin motivated glucose transporter-4 (GLUT4) translocation to the plasma membrane and diminished protein expression of glycogen synthase (5). Furthermore, estrogen therapy is related with rises in intracellular cyclic adenosine monophosphate (cAMP), protein kinase A (PKA) and phosphorylation of extracellular signal-regulated protein kinase (ERK1/2) proteins in pancreatic β-cells (7).

Genistein is an isoflavone in legumes and several herbal medicines. It is well-known as a natural estrogenic agent and is an inhibitor of tyrosine kinase. It has also been widely acknowledged for its potential hypolipidemic, anti-oxidative and anti-apoptotic effects. Human or animal studies have revealed that administration of isoflavones containing soy protein improved hyperglycemia (8, 9). Emerging studies showed that administration of isoflavone or genistein reduced plasma glucose levels in diabetic animals and post-

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menopausal women (10, 11), thus indicating an anti-diabetic role for genistein.

The aim of this study was to investigate the effects of genistein treatment on pancreas injury, β-cell, and islet viability in the animal model with estrogen deficiency. So, in the present study, we examined the effect of genistein on PI3K/AKT and mitogen-activated protein kinase (MAPK) and apoptosis signaling pathways as cell survival, inflammation, and cell death, respectively in ovariectomized diabetic rats.

Materials and Methods

Chemicals

Streptozotocin (STZ) and genistein were purchased from Sigma (St. Louis, Mo, USA). Polyclonal rabbit anti-ERK1/2 (sc-292838), anti-p-ERK1/2 (sc-16981-R), anti-AKT1(sc-1618), anti-p-AKT1(sc-135650), anti-caspase 3(sc-7148), anti-bcl2(sc-783), and HRP-conjugated goat anti-rabbit polyclonal antibody(sc-3837) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Animals

Forty Female Wistar Albino rats (weighing 180–220 g, about 10 weeks old) were obtained from the local breeding colony (animal laboratory of Neurosciences Research Center (NSRC), Tabriz University of Medical Sciences, Tabriz, Iran). The entire experimental approach was in compliance with the principles of the latest revised Guidelines of the National Institute of Health for the Care and Use of Laboratory Animals (NIH Publications No.80-23) and was approved by the ethical committee of Tabriz University of Medical Sciences (approval No.93/5-4/12). The rats were kept under the following controlled conditions: 12 hr: 12 hr light/dark cycle, 22–24 °C temperature, and 55%–65% humidity, with free access to water and a normal chow diet.

Study design

The animals were randomly divided into two groups including the ovariectomized (OVX, bilateral removal of ovaries (*n*=30)), and the sham-operated group (sham) (*n*=10). Surgery procedures were performed following anesthesia with ketamine (50 mg/kg, IP) and xylazine (5 mg/kg, IP). OVX rats were divided into three sub groups: control (OVX), OVX + diabetic (OVX.D), and OVX + D + genistein (OVX.D.G) (*n* = 10 per group). After surgical recovery (12), for diabetes induction in diabetic groups, high-fat diet (HFD) (25% protein, 58% fat, and 17% carbohydrate), was used *ad libitum* for 4 weeks. After 4 weeks HFD regimen, diabetic groups were subjected to injection of 30 mg/kg STZ (IP), which dissolved in 10 mM sodium citrate, pH 4.5, with 0.9% NaCl. Fasting blood glucose (FBG) levels were measured by using a glucometer after overnight fasting. FBG greater than 200 mg/dl in the diabetic group, were considered as the diabetic index (13). After confirmation of diabetes, rats in diabetic groups were fed a standard chow diet for 4 weeks. Genistein (Sigma-Aldridge, USA) was dissolved in DMSO+PEG (1 mg/kg in 500 µl of a mixture of DMSO (1.25%) and PEG-400 (98.75%)) and administrated (1 mg/Kg/day; SC.) for 8 weeks, simultaneously with the HFD regime (14). The rats in the sham group received the vehicle.

Biochemical assay

At the end of the experiment, FBG levels were measured to assess the onset of hyperglycemia (FBG > 200 mg/dl). Fasting plasma cholesterol, HDL, and triglyceride were evaluated using commercial diagnostic kits (Randox (UK)) in accordance with the manufacturer's instructions. Friedewald's formula was applied to calculation of serum levels of Low-density lipoprotein cholesterol (LDL-C) as follow: LDL-C_(in mg/dL) = TC_(in mg/dL) – (TG_(in mg/dL) /5) –HDL-C (in mg/dL) (15).

Immunoblotting analysis

Western blot analysis was performed to evaluate ERK1/2 and AKT phosphorylation and caspase-3 and Bcl-2 expression on pancreatic tissue. Briefly, snap-frozen pancreatic tissues were homogenized on ice in RIPA buffer supplemented with a protease inhibitor cocktail containing leupeptin, pepstatin, chymostatin, aprotinin, and antipain (5 µg/ml each) and were left for 20 min at 4 °C, and centrifuged at 12,000×g for 10 min at 4 °C. The supernatant was collected and stored at −80 °C. Proteins were separated by SDS-PAGE and electrophoretically transferred onto PVDF membranes. Nonspecific binding was blocked by 2 hr incubation of the membranes in 5% (w/v) nonfat dry milk in Tris-buffered saline (pH 7.5). Blots were then incubated for 2 hr at room temperature (or overnight at 4 °C) with primary antibodies (anti- ERK1/2, p-ERK1/2, AKT, p-AKT, caspase-3, and Bcl-2; Santa Cruz, USA) in the antibody buffer containing 1% (w/v) nonfat dry milk in TBS-T (0.05% (v/v) Tween-20 in Tris-buffered saline), then washed 3 times with TBS-T, and finally incubated for 1 hr with a secondary antibody (goat Anti-rabbit; Santa Cruz, USA) in the antibody buffer. Blots were developed for visualiza-tion using enhanced chemiluminescence (ECL) detection kit (Pierce, Rockford, IL). Band intensities on the immunoblots were quantified by densitometry using the Image j software.

Histological evaluation

After anesthesia, the pancreatic tissues were isolated and fixed in Bouin's solution for 72 hr, approximately. Then the tissues were dehydrated in ascending grades of ethanol (Merck, Germany). After dehydration, they were cleared and embedded in xylol and paraffin (Merck, Germany), respectively. At

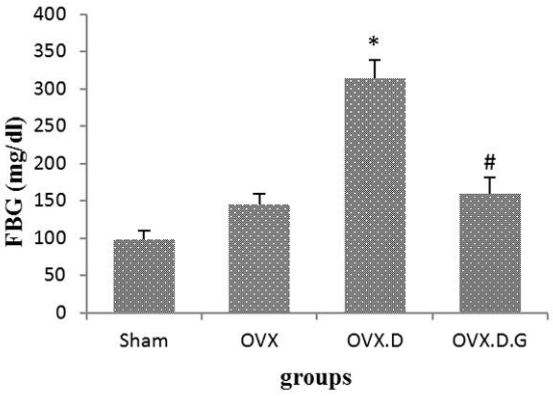

Figure 1. Fasting blood glucose levels in different studied groups OVX: ovariectomized, D: diabetic, and G: genistein-treated groups Data are expressed as mean±SEM
* *P*<0.05 vs. sham and OVX groups; # *P*<0.05 vs. OVX.D group

the end of these possesses, sections of 5 µm were obtained, stained with Hematoxylin-Eosin (H&E), and assessed under a light microscope (Olympus BH-2, Tokyo, Japan). Pancreas tissues were tested for morphological changes in islets of Langerhans.

Statistical analysis

All results of this study were described as the mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's multiple comparison post-test was used to determine differences between groups using SPSS program version 16.0. *P*<0.05 was considered statistically significant.

Results

Blood glucose levels

At the end of the experiment, we measured the FBG. Our results showed hyperglycemia in OVX.D rats during an overnight fasting (Figure 1). Genistein supplementation decreased glucose level in OVX.D.G rats compared to OVX.D animals (*P*< 0.05).

Biochemical analysis

The plasma lipid profiles are demonstrated in Table 1. Triglyceride (TG), total-cholesterol (t-Chol), and low-density lipoprotein cholesterol (LDL-C) levels were significantly higher in the OVX and OVX.D groups than those of the OVX.D.G and Sham operation groups (*P*<0.05). In addition, high-density lipoprotein cholesterol (HDL-C) in the OVX.D.G and sham operation groups were markedly higher than in the OVX.D and OVX groups (*P*<0.05).

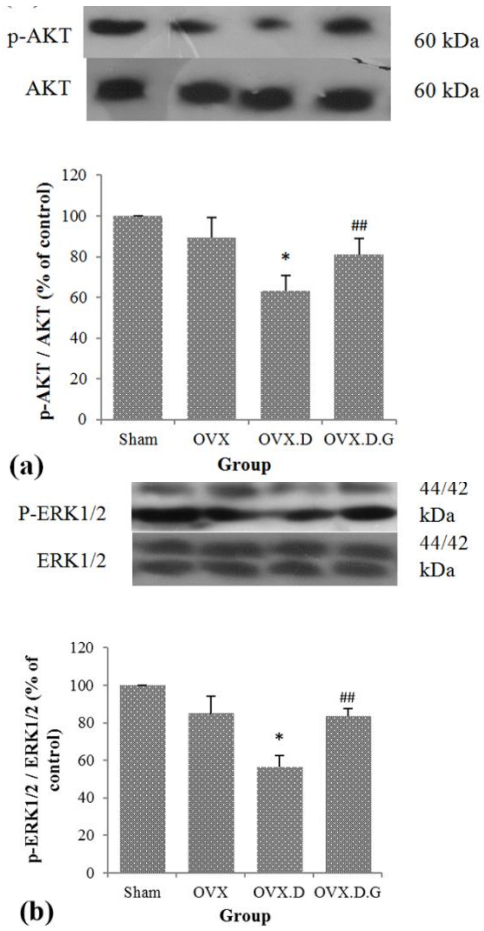

Figure 2. The hyperphosphorylation of AKT and ERK1/2 proteins by genistein treatment in the pancreas in studied groups
A and B immunoblotting images of p-AKT and p-ERK1/2, respectively. a; Quantitation of immunoblotting of p-AKT against total expression of AKT b: Quantitation of immunoblotting of p-ERK1/2 against total expression of ERK1/2. Data are shown as the means ± SEM of 5–6 individual experiments
* *P*<0.05 vs. sham group; ## *P*<0.05 vs. OVX.D groups

Table 1. Plasma lipid profiles in different studied groups

Group	TG mg/dl	T-Chol mg/dl	LDL-C mg/dl	HDL-C mg/dl
Sham	71.51±8.34	66.10±9.89	26.58±5.07	54.27±10.36
OVX	110.37±10.79*	104.54±12.33*	49.76±7.63*	43.78±8.11
OVX.D	131.09±13.18*	128.41±9.29*	68.32±8.82*	27.61±5.31*
OVX.D.G	98.12±11.73#	90.90±10.38#	41.48±6.09#	44.41±7.03#

TG: triglyceride; T-Chol; total cholesterol. LDL-C; low-density lipoprotein cholesterol. HDL-C; High-density lipoprotein cholesterol. OVX: ovariectomized, D: diabetic and .G: genistein-treated groups respectively. Data are expressed as mean±SEM
* *P*<0.05 vs. sham group; # *P*<0.05 vs. OVX.D groups

Effect of genistein on phosphorylation of AKT and ERK1/2 protein

Since both AKT and ERK have been implicated in cell growth, we tested whether genistein administration might affect these proteins. AKT and ERK 1/2 as markers of cell proliferation and growth were measured by Western blotting. Figure 2 shows the phosphorylation of AKT in the studied groups. The level of p-AKT protein was significantly decreased in OVX.D compared with the sham group (P<0.05). However, 2-months genistein administration significantly increased p-AKT in OVX.D.G compared to the ovariectomized diabetic group (Figure 2a). In addition, we concomitantly examined p-ERK1/2 protein levels in the pancreas tissues of all studied groups. The protein level of p-ERK1/2 was significantly decreased in OVX.D compared with the sham group (P<0.05). Similar to p-AKT levels, 2-month genistein administration significantly increased p-ERK1/2 in OVX.D.G compared to the ovariectomized diabetic group (Figure 2b).

Effect of genistein on Bcl-2 and caspase-3 protein levels

The present study showed that ovariectomy and induction of diabetes in rats led to a significant reduction in the mean value of pancreatic Bcl-2 levels (Figure 3a) compared to sham rats (P< 0.05). Genistein treatment significantly enhanced Bcl-2 levels, compared with the OVX.D group (P< 0.05). Also, our results showed that ovariectomy and diabetes in rats led to a significant increase in the mean value of pancreatic caspase-3 levels, a marker of cellular apoptosis, compared to sham rats (P< 0.05). As can be seen in Figure 3, genistein treatment significantly enhanced caspase-3 protein level (Figure 3b) in the pancreas (P< 0.05).

Effect of genistein on cell morphology in pancreas

Morphological evaluation of pancreatic tissue in the sham group showed normal size of the islets of Langerhans (Figure 4a). In the OVX group, atrophic size of the islets of Langerhans was evident. There was a few inflammatory cells and inflammation around the islet and sinusoid (Figure 4b). Induction of diabetes in ovariectomized rat (OVX.D) showed further atrophy and irregular size of the islets and leukocyte infiltration. Tissue injury signs such as vessels congestion and dilation, necrotic changes in Langerhans cells, cytoplasmic acidophilic degeneration, and amyloid depositions were evident in this group (Figure 4c). Treatment with genistein in the OVX.D.G group significantly decreased (Table 2) amyloid deposition and the inflammatory cells around the islet and sinusoid, compared to the OVX.D group (P<0.05) (Figure 4d).

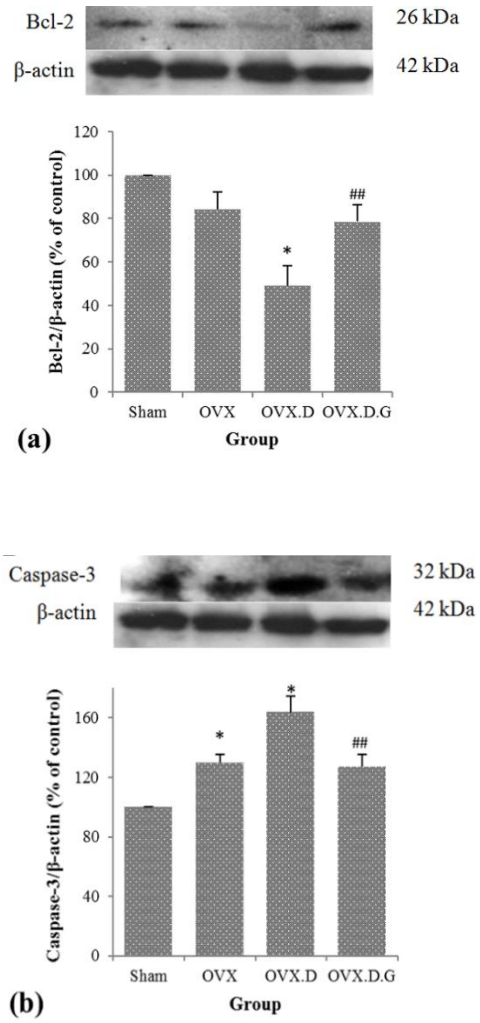

Figure 3. The up-regulation of Bcl-2 and down-regulation of caspase-3 proteins by genistein treatment, in the pancreas in studied groups.
A and B immunoblotting images of Bcl-2 and caspase-3, respectively. a; Quantitation of immunoblotting of Bcl-2 against expression of β-actin b: Quantitation of immunoblotting of caspase-3 against β-actin. Data are shown as the means±SEM of 5–6 individual experiments
* P<0.05 vs. sham group; ## P<0.05 vs. OVX.D groups

Table 2. Histological changes in pancreas in the studied group (H-E)

Group	inflammatory cells	Number of necrotic cells	Amyloidosis	Sinusoidal varicosity
Sham	0.25±0.07	0.03±0.01	0.0	0.09±0.04
OVX	0.59±0.19	0.56±0.13	0.81±0.22	1.09±0.11
OVX.D	4.7±0.41*	4.39±0.38*	5.11±0.54*	4.68±0.53*
OVX.D.G	2.86±0.25#	2.79±0.21#	2.71±0.24#	2.21±0.29#

A minimum of 10 fields in each pancreas slide were obtained and stained with H&E to evaluate severity of tissue injury (n = 7 for each group). Data are shown as the mean±SEM of 7 individual experiments
* P< 0.05 vs. sham group; # P<0.05 vs. OVX.D group

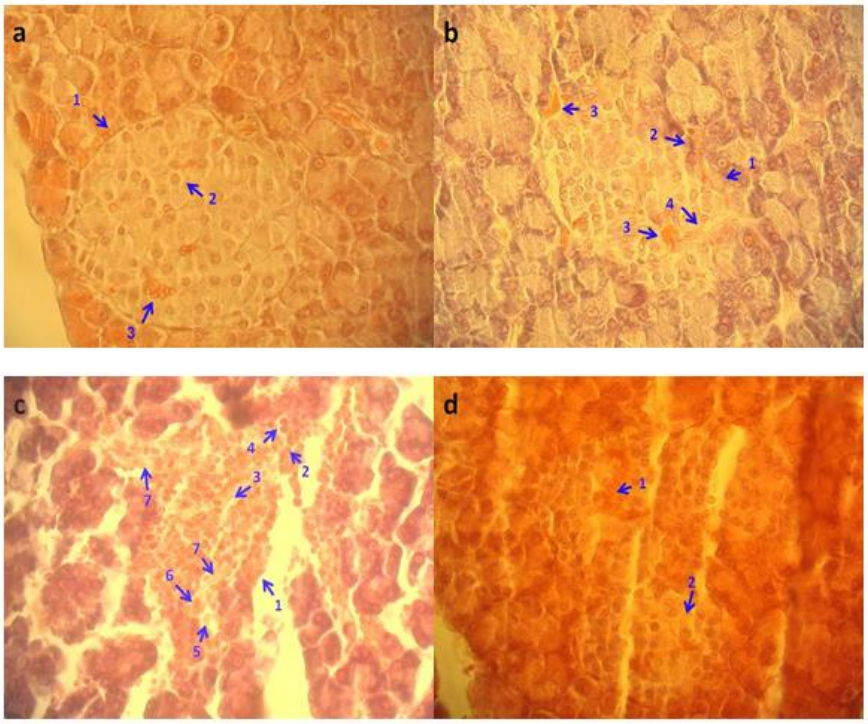

Figure 4. Morphological evaluation of pancreatic tissue: sham (a) normal size of the islets (1), islet cells nuclei (2), sinusoids containing erythrocytes (3). In OVX group (b) relative atrophy size (1), few inflammatory cells (2), sinusoidal varicosity (3), and rarely amyloid deposition (4) were observed. Morphological study in OVX.D (c) showed atrophy and irregular size in endocrine cells (1), leukocyte infiltration (2) together with vessels congestion (3), evident necrotic changes (4), vacuolar degeneration (5), acidophilic cytoplasm (6), and islet amyloid depositions (7). Treatment with genistein in OVX.D.G group (d) showed relatively atrophied size (1), decreased of inflammatory cells (2). (magnification of all images × 40)

Discussion

The points of the present study could be summarized as follows: Genistein 1) decreased plasma FBG, 2) ameliorated pancreatic abnormalities, 3) Upregulated ERK1/2 AKT and Bcl-2 proteins, and 4) down regulated caspase-3. This evidence suggests that the beneficial effects of genistein in pancreatic tissue regeneration may be partially mediated by inhibition of apoptosis via down-regulation of caspase-3 and promotion of cell survival via induction of the PI3K/AKT pathway. Diabetes mellitus is common in postmenopausal women and is the main risk factor for cardiovascular disorders, the principal cause of death in women in industrialized countries (2). The postmenopausal years are associated with a rise in fasting glucose and insulin levels, and with increased visceral adiposity (1). The significant decline in endogenous estrogen production following menopause or ovariectomy results in raised relative androgenicity and changes in body composition, that influence pancreatic β-cell function, hepatic glucose output, and insulin-induced glucose transport (5). In addition, evidence has shown that nonclassical estrogen membrane receptor initiates rapid differential actions in the endocrine pancreas.

In the current study, the results showed that ovariectomy increased FBG, and subsequent STZ injection further elevated hyperglycemia but genistein could markedly control and decrease hyperglycemia. Previous studies have shown that blood glucose concentration increases in diabetic control rats, but in genistein treated diabetic rats, blood glucose level is markedly decreased compared with diabetic rats (14, 16). It seems that the hypoglycemic effects of genistein are due to the improved level of serum insulin and the induction of peripheral metabolism of glucose (17).

Given that estradiol as a predominant form of adult estrogen has an anti-apoptotic role in β-cells that are mediated by the estrogen receptor-α (ERα). On the other hand, cross-talk between ovariectomy and diabetes has been already demonstrated. However, it is unclear what instigates augmented rate of apoptosis in β-cells during the pathogenesis of ovariectomy linked to type 2 diabetes although, both chronic exposure to raised levels of fatty acids and continued fluctuations of high circulating glucose levels, have a marked influence (18). This study have shown that loss of β-cell mass and function is essential to the development of both type 1 and 2 diabetes. Therefore, stimulation of β-cell proliferation is one of the critical strategies to prevent diabetes (18). Therefore, maintaining β-cell survival is a critical factor for preventing the onset of type 2 diabetes. The major cell-survival-related intracellular signaling pathway is the PI3K/AKT/mTOR (mechanistic target of rapamycin)

pathway, which is directly related to cellular proliferation, quiescence, and longevity. PI3K activation phosphorylates and activates AKT, localizing it in the plasma membrane. Phosphorylated AKT regulates a number of down-stream cell survival pathways such as activating cAMP response element binding protein (CREB) and localizing Forkhead box (FOXO) in the cytoplasm. In addition, there are many well-known factors that promote the PI3K/AKT pathway such as insulin which are inducers for activated ERK1/2, the most widely expressed members of the MAP kinase family. Interestingly, the importance of estrogens in modulating rapid signaling effects that act on these targets has been highlighted (19, 20). There is evident that AKT activation plays a central role in β-cell survival and has several anti-apoptotic substrates. Expression of AKT in β-cells inhibits FFA-induced apoptosis. Furthermore, transgenic expression of the AKT protects against STZ-induced diabetes and increases β-cell mass by extending β-cell survival and increasing β-cell size (18). Our result revealed that ovariectomy together with diabetes causes a marked decrease in p-AKT, p-ERK1/2, and Bcl-2, and an increase in caspase-3 protein, which is a DNA fragmentation-related enzyme. All of these signaling changes can lead to the pancreas tissue injury that demonstrated in the histological evaluation of pancreas tissue (see Figure 4).

Here we provided evidence that genistein improves rat islet β-cells by increasing the expression of p-AKT and p-ERK1/2. In addition, genistein reduced caspase-3 as final biomarker of apoptosis and increased anti-apoptotic Bcl-2 in the pancreas. Our results demonstrated that genistein can act as a growth factor for β-cells and provided an anti-diabetic effect for this compound. It was previously shown that oral administration of genistein (50 mg/kg BW/day) activated PI3K/AKT signaling in islet β-cells (21). Also, acute glucose may regulate β-cell survival and growth with ERK activation (22). For the first time, we showed that genistein stimulated ERK1/2 phosphorylation in the pancreas of ovariectomized diabetic rats. Activated ERK1/2 plays an essential role in environ-mentally induced cellular responses, comprising cellular proliferation, differentiation and growth. Such a prolonged ERK1/2 activation may be required for growth factors to drive β-cell proliferation (23). The effect of genistein on β-cell proliferation was not dependent on estrogen receptors. The molecular or pharmacological intervention of ERK1/2 eliminated genistein-stimulated β-cell proliferation, indicating that ERK1/2 is essential for genistein action. Genistein induces cAMP/PKA signaling and subsequent ERK1/2 phosphorylation in islets. Dietary intake of genistein markedly improves hyperglycemia, blood insulin levels and glucose tolerance in STZ-induced diabetic mice, associated with improved islet β-cell proliferation, mass, and survival (24). The similar finding in our study showed that administration of genistein markedly improves hyperglycemia in genistein-treated ovariectomized diabetic rats. These results showed that genistein might be a natural anti-diabetic compound by directly improving pancreatic injury through activation of the ERK1/2 signaling pathway.

Caspase-3 is the main effector involved in apoptotic pathways. Although evidence supports the importance of cell death in β-cells in the pathogenesis of diabetes, the particular role of caspase-3 in this procedure is unidentified (25). It is revealed that inhibition of the PI3K/AKT pathway is related with raised caspase-3, and activated AKT inhibits activation of caspase-3 (26). Moreover, the inhibition of the AKT pathway decreases Bcl-2 and Bcl-xL and increases the pro-apoptotic Bax (27). On the other hand, exposure of beta-cells to genistein activates PI3K/AKT signaling, up-regulates anti-apoptotic protein Bcl-2 expression, exerts an anti-diabetic effect, and promotes beta-cell survival (21). In addition, animal studies have shown that genistein administration inhibits caspase-3 activity, which allows for the possibility that genistein could inhibit apoptosis (28). Our results showed a reduction in Bcl-2 and increased caspase-3 in ovariectomized diabetic rat. Two months genistein treatment reversed these effects and improved pancreas damage and glucose homeostasis. These findings suggest that the increased Bcl-2 and reduction of caspase-3 might be one of the mechanisms by which genistein protects pancreatic β-cells from apoptosis.

Conclusion

In summary, we found that genistein may be a protective factor for Langerhans islet β-cells by targeting the AKT and ERK1/2 expression and preserving pancreas via decreasing caspase-3 and increasing anti-apoptotic Bcl-2 protein levels. Our animal studies demonstrated that genistein is capable of preserving islet β-cell mass and improving diabetes in ovariectomized diabetic rat. In this context, these findings may potentially lead to the development of novel, natural agents for diabetes prevention and treatment in postmenopausal women.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Carr MC. The emergence of the metabolic syndrome with menopause. JCEM 2003; 88:2404-2411.
2. Brand JS, van der Schouw YT, Onland-Moret NC, Sharp SJ, Ong KK, Khaw K-T, *et al.* Age at menopause, reproductive life Span, and Type 2 diabetes risk results from the EPIC-InterAct study. Diabetes Care 2013; 36:1012-61019.
3. Kim C, Edelstein SL, Crandall JP, Dabelea D, Kitabchi AE, Hamman RF, *et al.* Menopause and risk of diabetes in the Diabetes Prevention Program. Menopause 2011; 18:857.
4. Bonds D, Lasser N, Qi L, Brzyski R, Caan B, Heiss G, *et al.* The effect of conjugated equine oestrogen on diabetes incidence: the Women's Health Initiative randomised trial. Diabetologia 2006; 49:459-468.
5. Appiah D, Winters SJ, Hornung CA. Bilateral oophorectomy and the risk of incident diabetes in postmenopausal women. Diabetes Care 2014; 37:725-733.
6. Pirimoglu Z, Arslan C, Buyukbayrak E, Kars B, Karsidag Y, Unal O, *et al.* Glucose tolerance of premenopausal women after menopause due to surgical removal of ovaries. Climacteric 2011; 14:453-457.
7. Gilbert ER, Liu D. Anti-diabetic functions of soy isoflavone genistein: mechanisms underlying its effects on pancreatic β-cell function. Food Funct 2013; 4:200-212.
8. Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA, *et al.* Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. Diabetes Care 2002; 25:1709-1714.
9. Lavigne C, Marette A, Jacques H. Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. Am J Physiol Endocrinol Metab 2000; 278:E491-E500.
10. Park SA, Choi MS, Cho SY, Seo JS, Jung UJ, Kim MJ, *et al.* Genistein and daidzein modulate hepatic glucose and lipid regulating enzyme activities in C57BL/KsJ-db/db mice. Life Sci 2006; 79:1207-1213.
11. Cheng SY, Shaw NS, Tsai KS, Chen CY. The hypoglycemic effects of soy isoflavones on postmenopausal women. J Womens Health (Larchmt) 2004; 13:1080-1086.
12. Habibi P, Alihemmati A, NourAzar A, Yousefi H, Mortazavi S, Ahmadiasl N. Expression of the Mir-133 and Bcl-2 could be affected by swimming training in the heart of ovariectomized rats. Iran J Bacic Med Sci 2016; 19:381-387.
13. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res 2007; 125:451.
14.Yousefi H, Alihemmati A, Karimi P, Alipour MR, Habibi P, Ahmadiasl N. Effect of genistein on expression of pancreatic SIRT1, inflammatory cytokines and histological changes in ovariectomized diabetic rat. Iran J Bacic Med Sci 2017; 20:423-429.
15. Nikasa M, Karimi P, Rajavand H, Afshari F, Jafarlou M, Soltanali M. High cholesterol diet increases expression of cholesterol 24-hydroxylase and BACE1 in rat hippocampi: implications for the effect of diet cholesterol on memory. IRCMJ 2016.
16. Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life Sci 2006; 79:1578-1584.
17. Skim F, Lazrek H, Kaaya A, El Amri H, Jana M. Pharmacological studies of two antidiabetic plants: Globularia alypum and Zygophyllum gaetulum. Therapie 1998; 54:711-715.
18. Dickson LM, Rhodes CJ. Pancreatic β-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? Am J Physiol Endocrinol Metab 2004; 287:E192-E198.
19. Acconcia F, Kumar R. Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Lett 2006; 238:1-14.
20. Acconcia F, Ascenzi P, Bocedi A, Spisni E, Tomasi V, Trentalance A, *et al.* Palmitoylation-dependent estrogen receptor α membrane localization: regulation by 17β-estradiol. Mol Biol Cell 2005; 16:231-237.
21. Wang A, Liu D, Luo J, Suh KS, Moore W, Alkhalidy H. Phytochemical genistein promotes pancreatic beta-cell survival and exerts anti-diabetic effect via GPR30-mediated mechanism (1045.44). FASEB J 2014; 28:1045.1044.
22. Wijesekara N, Krishnamurthy M, Bhattacharjee A, Suhail A, Sweeney G, Wheeler MB. Adiponectin-induced ERK and Akt phosphorylation protects against pancreatic beta cell apoptosis and increases insulin gene expression and secretion. J Biol Chem 2010; 285:33623-33631.
23. Lingohr MK, Dickson LM, McCuaig JF, Hugl SR, Twardzik DR, Rhodes CJ. Activation of IRS-2—Mediated signal transduction by IGF-1, but not TGF-α or EGF, augments pancreatic β-Cell proliferation. Diabetes 2002; 51:966-976.
24. Fu Z, Zhang W, Zhen W, Lum H, Nadler J, Bassaganya-Riera J, *et al.* Genistein induces pancreatic β-cell proliferation through activation of multiple signaling pathways and prevents insulin-deficient diabetes in mice. Endocrinology 2010; 151:3026-3037.
25. Liadis N, Murakami K, Eweida M, Elford AR, Sheu L, Gaisano HY, *et al.* Caspase-3-dependent β-cell apoptosis in the initiation of autoimmune diabetes mellitus. Mol Cell Biol 2005; 25:3620-3629.
26. Duxbury MS, Ito H, Benoit E, Zinner MJ, Ashley SW, Whang EE. RNA interference targeting focal adhesion kinase enhances pancreatic adenocarcinoma gemcitabine chemosensitivity. Biochem Biophys Res Commun 2003; 311:786-792.
27. El-Rayes BF, Ali S, Ali IF, Philip PA, Abbruzzese J, Sarkar FH. Potentiation of the effect of erlotinib by genistein in pancreatic cancer: the role of Akt and nuclear factor-κB. Cancer Res 2006; 66:10553-10559.
28. Aras AB, Guven M, Akman T, Alacam H, Kalkan Y, Silan C, *et al.* Genistein exerts neuroprotective effect on focal cerebral ischemia injury in rats. Inflam 2015; 38:1311-1321.