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Published in:
Life Sciences

DOI:
10.1016/j.lfs.2020.117969

Publication date:
2020

Life Sciences 256 (2020) 117969

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Cannabinoids as anti-ROS in aged pancreatic islet cells

Maryam Baeeri[a,1], Mahban Rahimifard[a,1], Seyed Mojtaba Daghighi[a], Fazlullah Khan[a], Seyed Alireza Salami[b], Shermineh Moini-Nodeh[a], Hamed Haghi-Aminjan[c], Zahra Bayrami[a], Farhad Rezaee[d,e], Mohammad Abdollahi[a,*]

[a] Toxicology and Diseases Group, Pharmaceutical Sciences Research Center (PSRC), The Institute of Pharmaceutical Sciences (TIPS), Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS), Tehran, Iran
[b] Department of Biotechnology, University of Tehran, Tehran, Iran
[c] Pharmaceutical Sciences Research Center, Ardabil University of Medical Sciences, Ardabil, Iran
[d] Department of Cell Biology, University of Groningen, Groningen, the Netherlands
[e] Department of Gastroenterology-Hepatology, Erasmus Medical Center, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Insulin
Oxidative stress
ROS
Islet cells
Cellular aging
Cannabidiol

ABSTRACT

Aims: Cannabinoids are the chemical compounds with a high affinity for cannabinoid receptors affecting the central nervous system through the release of neurotransmitters. However, the current knowledge related to the role of such compounds in the regulation of cellular aging is limited. This study aimed to investigate the effect of cannabidiol and tetrahydrocannabinol on the function of aged pancreatic islets.

Main methods: The expression of *p53*, *p38*, *p21*, *p16,* and *Glut2* genes and β-galactosidase activity were measured as hallmarks of cell aging applying real-time PCR, ELISA, and immunocytochemistry techniques. Pdx1 protein expression, insulin release, and oxidative stress markers were compared between young and aged rat pancreatic islet cells.

Key findings: Upon the treatment of aged pancreatic islets cells with cannabidiol and tetrahydrocannabinol, the expression of *p53*, *p38*, *p21* and the activity of β-galactosidase were reduced. Cannabidiol and tetrahydrocannabinol increase insulin release, Pdx1, *Glut2,* and thiol molecules expression, while the oxidative stress parameters were decreased. The enhanced expression of Pdx1 and insulin release in aged pancreatic islet cells reflects the extension of cell healthy aging due to the significant reduction of ROS.

Significance: This study provides evidence for the involvement of cannabidiol and tetrahydrocannabinol in the oxidation process of cellular aging.

1. Introduction

Cellular aging is a process affected by various factors, leading to cell survival decrement and may cause a reduction in different tissues and organs' physiological activities [1]. Although the term cellular aging is used in all biological processes, there are two distinct cellular aging types or phases; 1- mature or healthy cellular aging and 2- premature cellular aging. Both aging phases lead to different diseases, one can occur in late, and another arises in the early phase of the aging process. There are numerous underlying factors involved in the aging process such as genetic damage, DNA methylation alterations, post-transitional modification and folding of proteins, production of reactive oxygen species (ROS), weakening of the immune system and inflammation [2]. Despite the recent advances in scientific researches, the precise mechanisms associated with healthy cellular aging [3] process and premature cell age-related disorders including type 2 diabetes (T2D), cancer, osteoporosis, and Alzheimer's are still unknown [4].

Although cellular aging is an ignorable risk factor for various metabolic disorders [5], the cellular metabolic function will be reduced with aging, and it can increase the occurrence of metabolic diseases such as T2D. It must be noticed that this type of diabetes is predominant in aged people (healthy aging of cells) as compared to adults [6]. The decrease in the propagation of β-cells in the pancreatic islets of Langerhans over time can occurr due to the failure of age-related mitotic signal transduction pathways. In this regard, the *p38* mitogen-activated kinase pathway (MAPK) signals can affect the expression of cyclin-

* Corresponding author at: The Institute of Pharmaceutical Sciences (TIPS) and School of Pharmacy, Tehran University of Medical Sciences, Tehran 1417614411, Iran.
E-mail address: Mohammad@TUMS.Ac.Ir (M. Abdollahi).
[1] Equally as the first author.

https://doi.org/10.1016/j.lfs.2020.117969
Received 15 April 2020; Received in revised form 10 June 2020; Accepted 11 June 2020
Available online 15 June 2020

dependent kinase 1 (CDK1) in the endocrine cell aged islets [7]. A disruption in the *p38* MAPK signaling transduction in the aged mice decreases the expression of *p16*INK4a, *p19*arf, and CDK1 in response to the propagation of β-cells. This effect may be neutralized by *p53*-induced phosphatase 1. High levels of this phosphatase diminish the expression of *p16*INK4a and hence improves the regeneration potential of β-cells after their impairment by streptozotocin [7].

The cellular senescence is an irreversible seizure of the cell division which occurs after DNA damage, generation of oxidative stress, the erosion of telomerase, or the activation of an oncogene [8]. There are essential proteins such as *p16*, *p53*, and *p21,* which act as inhibitors of the cell cycle and, in this way, take part in the cellular senescence process [9]. The proliferation of β-cells and the generation of ROS accelerate cellular senescence [10]. It has been proved that large size islets are more frequently present in the young rats as compared to the aged rats. Furthermore, pancreatic β-cells *vs.* islet region ratio decrease expressively with age. Such findings indicate that *p16*, *p53*, and *p21* are the senescence markers that were overexpressed, and the generation of oxidative stress increased as a result of ROS generation and was similarly down-regulated in the eNOS in the aged rats [11]. In this regards, glucose transporter 2 (*Glut2*), the main glucose transporter in pancreatic β-cells regulates glucose balance as a result of its robust glucose sensing and transport capability [12]. However, it is indicated that the lack of *Glut2* expression leads to the release of insulin and hyperglycemia, which may affect β-cells function, causing T2D. This reduction in *Glut2* expression levels in aged islets of rodents has demonstrated a strong correlation between down-regulation of *Glut2* and cellular aging [13].

Regarding the previous studies, cell aging is linked with the development of oxidative stress and the generation of free radicals, and such factors can indeed exert their impact on a set of molecular pathways [14]. Hence, the application of compounds with the ability to decrease the adverse effects of oxidative stress can be considered as useful candidates for the treatment of aging and its associated disorders. In this regard, cannabinoids compounds, which contribute to a decrease of oxidative stress and inflammation [15], have gained increasing attention due to their antioxidant capacity and therapeutic role. There are > 100 known cannabinoids that can be obtained from *Cannabis sativa* L. Among different cannabinoids, cannabidiol (CBD) and tetrahydrocannabinol (THC) are the most abundant cannabinoids originated from cannabis. These two cannabinoids act together on the endocannabinoid system with biological effects [16]. In this study, we used the aged rat pancreatic islets cells to investigate the anti-aging and anti-diabetic effects of CBD and THC on the functions of endocrine cells produced by pancreatic islets of Langerhans. To this end, different features of CBD and THC, such as their anti-aging, functional and antioxidant effects on the young and aged islets were evaluated.

2. Materials and methods

2.1. Chemicals and islets isolation

Rat-specific enzyme-linked immunosorbent insulin ELISA kit was obtained from Mercodia (Sweden). CBD and THC were purchased from Cerilliant (Round Rock, TX). Similarly, the mouse anti-Pdx1 and rabbit anti-insulin were purchased from Santacruz biotechnology Inc. (USA). Goat Anti-Mouse IgG Fc (DyLight® 650) and Goat Anti-Rabbit IgG H&L (FITC) were purchased from Abcam (United Kingdom). All other utilized materials and chemicals were purchased from Sigma-Aldrich (GmbH Munich, Germany).

The ethics committee approved all procedures regarding use of animals for this research in Tehran University of Medical Sciences (#IR.TUMS.VCR.REC.1397.1053). Animal experiments were performed in male Wistar rats, aged 8 and 72 weeks as young and aged animals, respectively. At the initial phase, rats were housed in separately ventilated cages with free access to food and water in the animal laboratory for adequate time to adapt to the new environment. Groups of ten rats (age 72 weeks) were randomly assigned to three groups of aged islets, aged islets + CBD, and aged islets + THC while one group of ten rats (age eight weeks) was subjected to young islets as control. For islet isolation, rats were anesthetized using ketamine-xylazine injection with a ratio of 10:1 (100 mg/kg ketamine/10 mg/kg xylazine). After sacrifice, to gain access to the abdominal cavity, laparotomy was done, removing lymph nodes, fats, and vessels. The removed pancreas was washed with buffer Krebs at pH = 7, after which the tissues were cut into pieces on ice, and then centrifuged two times at 3000*g* for 60 s. Next, collagenase was added at 37 °C in order to remove surrounding tissues, and then bovine Serum Albumin (BSA) was added to block the process. Islet cells with 100–150 μm in size were carefully chosen using a stereomicroscope and cultured in standard RPMI 1640 medium containing 10% fetal bovine serum, 1% penicillin-streptomycin solution, and 8.3 mmol/l glucose. After incubation of the cells in the presence of logarithmic concentrations (0.1, 1, 10 and 100 μM) of CBD and THC at 37 °C with 5% CO_2 for 24 h [17], the following assessments were performed in triplicate.

2.2. Safety determination of CBD and THC

The safety of CBD and THC for finding EC_{50} was investigated by MTT assay. This assay is based on the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium, in which in viable cells the mitochondrial respiration changes the color of tetrazole from yellow to purple. In this regard, aged islet cells in the presence of logarithmic concentrations (0.1, 1, 10 and 100 μM) of CBD and THC were incubated for 24 h and then were centrifuged and washed twice with phosphate buffer. Following these steps, cells received 50 μl of MTT and were incubated for four h at 37 °C and 5% CO_2 humidified atmosphere. Upon addition of 150 μl of dimethyl sulfoxide (DMSO), the solution was measured at absorbance 570 nm. In this experiment, the viability is presented as the percentage of control and the EC_{50} of CBD and THC in aged islets was measured.

2.3. In vitro evaluation of islet cells function

Insulin release of islets treated with ten μM CBD and THC as used for evaluation of islets function was measured using an ELISA kit (Mercodia, Sweden) according to the manufacturer's procedure. To this end, both aged and young pancreatic islets were incubated with ten μM CBD and THC for 24 h at 37 °C. After this period, the islets were washed with Krebs buffer and centrifuged at 3000 *g* for 1 min, after which the supernatants were removed. Next, incubation by 2.8 mM glucose was done for 30 min, and the cells were divided into two groups, one of them received 2.8 mM glucose (the basal dose) while the second group was treated with 16.7 mM glucose (stimulant dose). After 1 h, cells were centrifuged, and the supernatants were collected. To measure proteins concentration, the absorbance was determined at 595 nm using the Bradford protocol. BSA was utilized as the standard.

2.4. Immunostaining

After 24 h treatment with CBD and THC, pancreatic islet cells were fixed in 4% formaldehyde for 2 h. The fixed islets were embedded in paraffin and separated into 7 μm-thick sections. The Paraffin-embedded slices were rehydrated, and antigen retrieval was completed using a Thermo Scientific™ PT Module (Greece Co.). In this step, rabbit anti-insulin (1:200; sc-9168), and mouse anti Pdx1 (1:150; sc-390,792) were used as primary antibodies. The secondary antibodies were conjugated to FITC [1:500; goat anti-rabbit IgG (H&L); ab6717; abcam] or DyLight 650 [1:200; Goat Anti-Mouse IgG Fc; ab97018; abcam]. The nuclear counterstain, 4′6′-diamidino-2-phenylindole (DAPI), was also used. All experiments repeated at least three times. The histological sections were investigated blindly by the independent reviewer using a

fluorescence microscope (Olympus BX51). The slides were subsequently analyzed using Image-Pro Plus version 6.0 image analysis software (Media Cybernetics, Rockville, MD, USA). According to the immune-density, the pancreatic islet areas marked with insulin and Pdx1 were selected, and the percentage of positive area for each antibody was determined as described previously [17].

2.5. Evaluation of cellular aging-associated biomarkers

Cellular aging-associated biomarkers; *p53*, *p38*, *p21*, *p16*, *Glut2* genes, and SA-β-gal activity were determined using qRT-PCR and relevant assay kit, respectively. Total RNA was extracted from all pancreatic islets using the TRIZol® reagent kit according to manufacturers' protocol. The concentration of RNA was measured using a NanoDrop 2000c UV–vis spectrophotometer (Thermo Fisher Scientific, CA). The iScript cDNA Synthesis kit was used to make cDNAs, and Glyceraldehyde 3-Phosphate dehydrogenase (GAPDH) was used as an internal control [18]. The Q-RT-PCR assessment was performed on a light cycler 96 system (Roche, Germany) by SYBER Green Master Mix. The relative gene expression was analyzed using $2^{-\Delta\Delta Ct}$ formula. The results were normalized against GAPDH. The abbreviations, accession numbers, and primers sequence are shown in Table 1.

SA-β-gal activity as one of the critical cellular aging-associated biomarkers was measured in both aged and young islets in the presence and absence of CBD and THC using β-galactosidase ELISA kit (Cusabio, China) according to the manufacturer's instructions. The absorbance was measured at 450 nm using ELISA reader.

2.6. Evaluation of oxidative stress responses

2.6.1. Total thiol molecules assay

For the estimation of total thiol molecules (TTM) as an essential target of the oxidant signal, 200 μl of Tris-EDTA solution was added to ten μl of the all prepared samples. Supernatants were collected, and 40 mM EDTA with pH 8.2 was added and vortexed. Then, samples were mixed with 4 μl of 5–5′-dithiobis-2-nitrobenzoic acid followed by a 15-min incubation at room temperature. Finally, the absorbance of samples was determined at 412 nm. Final data are shown as mM, as described previously [19].

2.6.2. ROS assay

2′,7′-dichlorofluorescein diacetate (DCFH-DA) a fluorogenic reagent was used to measure the production of ROS which after penetration into the cell it was converted to 2′,7′-dichlorodihydrofluorescein (DCFH) catalyzed by intracellular esterase and then oxidized by ROS into DCF. In this method, the absorbance of DCF production was measured every 5 min for 60 min by ELISA (Synergy HT, BioTek, VT, USA) (λ ex = 488 nm, λ em = 529 nm). For standardizing our measurements, the amount of total protein was used.

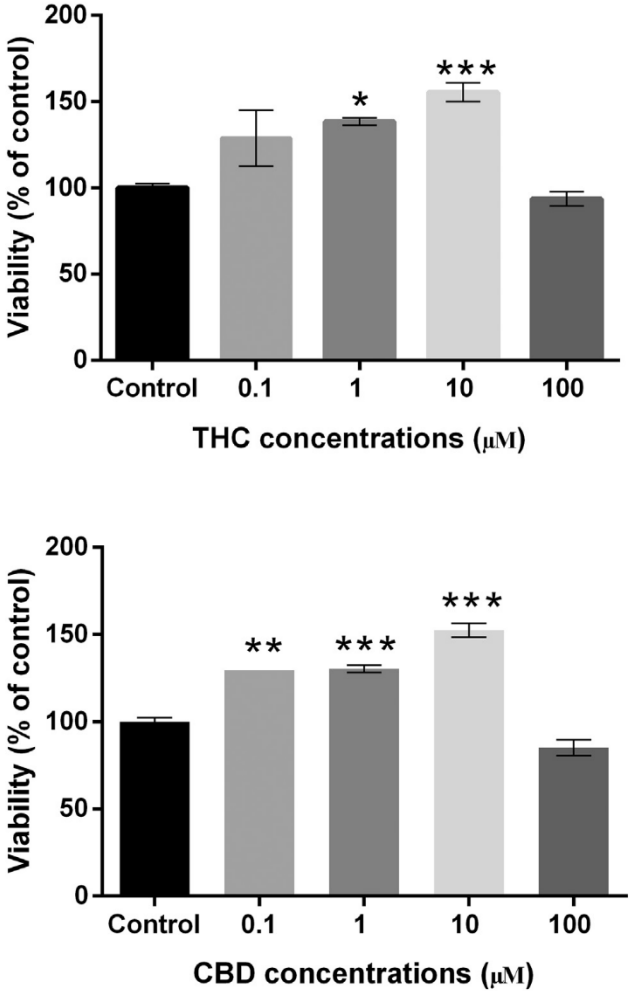

Fig. 1. Cell viability of pancreatic islets from aged Wistar rats in presence of logarithmic concentrations (0.1, 1, 10 and 100 μM) of cannabidiol (CBD) and tetrahydrocannabinol (THC) after 24 h of treatment. The values are expressed as mean ± SEM of islets viability percentage for each concentration, ($n = 10$). *, **, ***, significantly different from control group at $p < .05$; $p < 0.01$ and $p < .001$, respectively.

2.7. Statistical analysis

Data are presented as means with the standard error of the mean for three independent experiments and analyzed using Stats-Direct software version 3.1.122. To measure the correlation between data, One-way ANOVA and Tukey's multi-comparison assessment were used. The *P*-Values < .05 were considered statistically significant.

Table 1
The primers of genes used for RT-PCR.

Gene name	Gene symbol	Accession no. Primer	Primer sequence (5′-3′)
Tumor protein *p53*	*p53*	NM_030989.3	F: CCC TGA AGA CTG GAT AAC TG R: AAT TAG GTG ACC CTG TCG CT
Mitogen activated protein kinase 14	*p38α*	NM_031020.2	F: GAC ACC CCC TGC TTA TCT CA R: GAC ACC CCC TGC TTA TCT CA
Rattus norvegicus cyclin-dependent kinase inhibitor 2A	*p16*	NM_031550.1	F: CGATACAGGTGATGATGATG R: TACTACCAGAGTGTCTAGGA
Rattus norvegicus KRAS proto-oncogene	*p21*	NM_031515.3	F: AACCTGTCTCTTGGATATTCT R: GGACCATAGGCACATCTT
Solute carrier family 2 member 2	Slc2a2/*Glut2*	NM_012879.2	F: AGTCACACCAGCACATACGA R: TGGCTTTGATCCTTCCGAGT
Glyceraldehyde-3-phosphate Dehydrogenase	GAPDH	NM_017008.4	F: AGT CTA CTG GCG TCT TCA CC R: CCA CGA TGC CAA AGT TGT CA

3. Results

3.1. Cell viability

The percentage of viable islet cells treated with different concentrations of THC and CBD for 24 h is presented in Fig. 1. All used THC and CBD concentrations (0.1, 1, 10, and 100 μM) were in the range of physiological dose and did not lead to a cell viability reduction compared to the control. As shown in Fig. 1a and b, the application of both THC and CBD with following concentrations of 0.1, 1 and 10 μM resulted in a statistically significant increment in the viability of islet cells when compared to the percentage of viable cells in the control group. Importantly, the highest effect ($p < .001$) of both THC and CBD on enhancing of islet cell viability in comparison with control was observed in the concentration of ten μM. Although the trend of increment in the viability of islet cells was stopped receiving 100 μM of both THC and CBD, the application of 100 μM THC and CBD did not significantly lead to a cell viability reduction compared to the control, whereas 100 μM THC and CBD induced a significant cell viability reduction when compared to the physiological doses (0.1, 1 and 10 μM).

3.2. Insulin release

To examine the function of aged and young islets in the presence of ten μM THC and CBD, the insulin release was assessed in two stimulated (16.7 mM) and basal (2.8 mM) concentrations of glucose. As it is shown in Fig. 2, the aged islets in both levels of glucose have a significantly lower insulin release than young islets ($p < .05$). The aged islet cells treated with ten μM of CBD resulted in an increase of insulin release in both concentrations of glucose in comparison with young and aged islets without CBD treatment ($p < .01$ and $p < .001$ respectively). After treatment of the aged pancreatic islets with CBD, there also was a significant ($p < .01$) increment in insulin release in stimulated as compared to basal glucose concentration. Although the aged islets treated with CBD showed an apparent enhancement in the release of insulin, THC did not affect the insulin release from aged islets in comparison with young and aged islets without THC treatment.

3.3. Immunocytochemistry

The post-treatment levels of Pdx1 and insulin expression is displayed in Figs. 3 and 4. Immunostaining results indicated that the expression levels of insulin and Pdx1 were diminished in aged islet cells, comparing to the young islets (Fig. 3). The treatment of the islet cells by either CBD or THC caused a significant increase in the expression levels of insulin as compared to the aged islets cells (Fig. 4a). This difference also was observed in the level of Pdx1 expression between aged islets and aged islets stimulated with CBD and THC (Fig. 4b). Taken together, the results of the immunocytochemistry assay showed that the treatment of aged islet cell with CBD and THC elevates the expression of insulin and Pdx1 levels in aged islets.

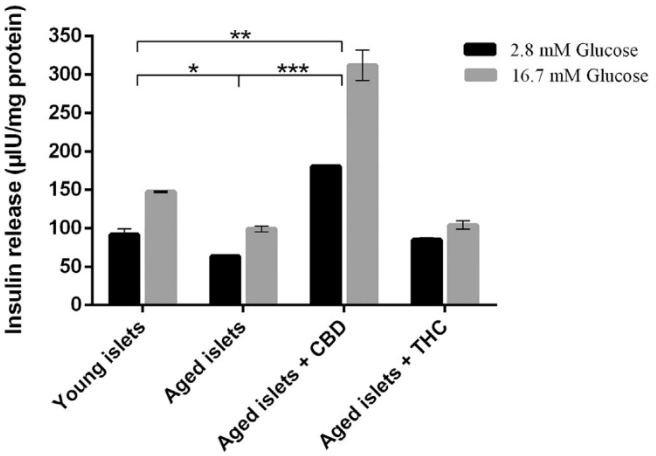

Fig. 2. Insulin release in pancreatic islets from Wistar rats after 24 h of treatment with ten μM of cannabidiol (CBD) and tetrahydrocannabinol (THC). Islet cells were treated for 1 h with 2.8 and 16.7 mM glucose as basal and stimulant concentrations, respectively. The values are expressed as mean ± SEM of 4 groups of islets for each case, ($n = 10$). *, **, ***, indicates significantly differences at $p < .05$; $p < 0.01$ and $p < .001$, respectively.

3.4. Cellular aging-associated biomarkers

Representative results of genes associated with cellular aging in pancreatic islet cells treated with ten μM of CBD and THC are presented in Fig. 5. The expression levels of all evaluated genes, i. e. *p53*, *p38*, *p21*, and *p16* genes in aged islets were significantly higher than young islets ($p < .001$). The expression of *p53* gene in aged islets treated with ten μM CBD was up and down-regulated as compared to young and aged islets respectively ($p < .01$ and $p < .001$). Upon the treatment of aged islets with THC, there was an apparent reduction of *p53* and *p38* genes expression as compared to aged islets with and without CBD treatment. Interestingly, *p21* and *p16* genes expression in islets cells treated with CBD showed a reduction as compared to aged islets without CBD treatment ($p < .01$ and $p < .001$). Taken together, treatment of aged islets with THC demonstrated that relative *p21* and *p16* expression in this group had a significant reduction in comparison with the aged pancreatic islets ($p < .01$).

Fig. 5. shows the expression of *Glut2* is down-regulated in aged islets compared to the young islets ($p < .01$). Although, after treatment of islets with both CBD and THC, the expression of *Glut2* was upregulated, the *Glut2* expression was significantly increased when the aged islets were treated with THC ($p < .001$). Moreover, as described in Table 2, aged islets receiving CBD and THC showed a significant decrease in β-galactosidase activity as compared to the group of aged islets without CBD and THC treatment.

3.5. Oxidative stress biomarkers

TTM and the percentage of ROS as oxidative stress biomarkers in pancreatic islets after treatment with ten μM of CBD and THC are presented in Fig. 6. The aged islets that have received both CBD and THC presented a significantly higher TTM than aged islets without CBD and THC treatment (Fig. 6a). There were no statistically significant differences in TTM values between the treated groups and the young islet group.

As depicted in Fig. 6b, the percentage of ROS in aged islets treated with CBD was significantly lower than both young and aged islets ($p < .001$). It was also observed that the amount of ROS was highly reduced in aged islets added THC as compared to both young and aged islets ($p < .001$).

3.6. Data correlation

Correlation analysis between *p53*, *p38*, *p21,* and *p16* expressions as aging biomarkers and Pdx1 and insulin levels in stimulating phase as functional markers, are shown in Table 3. As it is shown, most of the aging biomarkers show a significant positive correlation with each other. Also, Table 3 represents a negative and statistically significant correlation between the expression of Pdx1 protein in endocrine pancreatic islet cells and *p38* ($p < .05$), *p53,* and *p21* ($p < .01$).

4. Discussion

In every living organism and through the normal biological processes, protein, lipids, and DNA undergo oxidation processes. In the normal aging process and premature cell aging, the increment of the

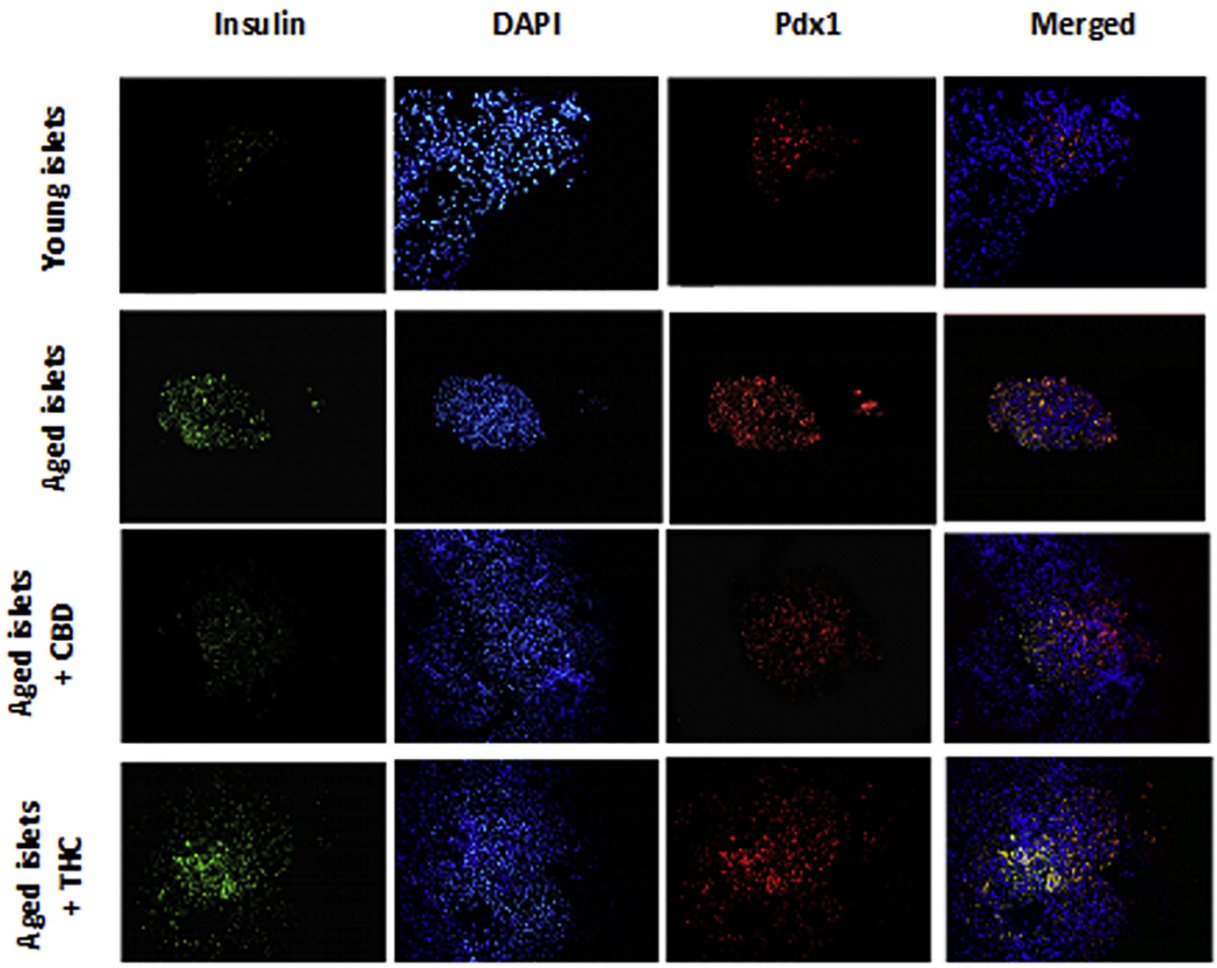

Fig. 3. Immunostaining of islet cells against insulin protein, DAPI, Pdx1 and the combination of the staining display in first, second, third and the last column respectively. The pictures were taken at 40 × magnification.

oxidation processes is obvious, because of exposure to environmental toxicants. This study was designed to gain more knowledge about the molecular mechanisms of the antioxidant capacity of CBD and THC and aging-induced disorders in refining the function of aged islets cells. As there exists a strong association between oxidative stress and cellular aging, we showed that CBD and THC could block the aging signaling pathways through the enhancement of antioxidant capability in the islets of the Langerhans cells.

The rise of oxidative stress parameters supports the physiological role of oxidative stress in ROS induced cellular aging in the pancreas [20]. The results obtained by the measurement of hallmarks of oxidative stress also confirm the role of ROS in this phenomenon.

In the current research, ROS as an oxidative stress biomarker was considerably higher in the islet cells obtained from the aged rats. In this regard, total thiol, which is known as a defense mechanism of the human body against free radicals [21], was significantly lower in the islet cells obtained from aged rats. Under normal physiological conditions, the macromolecules such as proteins, lipids, and DNA are exposed continuously to oxidation processes. At the same time, the formed free radicals are usually deactivated by the body antioxidant system [22]. Accordingly, the constant exposure to chemical agents may speed up macromolecules oxidation through the formation of ROS. These free radicals have severe adverse effects on proteins, lipids, and DNA. Therefore, the subsequent oxidative damages of these macromolecules may lead to several chronic disorders [23,24].

The pancreas oxidative defense system is partly insubstantial, and its Nucleus is more sensitive to the toxic effects of free radicals. The excessive production of ROS, followed by exposure to chemical agents, alters the insulin signaling pathways, which causes hyperglycemia [25]. The activity of β-galactosidase at the highest levels can diminish the insulin signaling pathways and hence causes insulin resistance in the adipose tissues, muscle cells, and, in turn, T2D [26].

The islets of Langerhans are responsible for endocrine action of the pancreas. Hence, in diabetes studies, to measure the toxicity of various chemical factors in terms of glucose metabolism, isolated islet cells are extensively used [27]. The absorption of glucose regulates the secretion of insulin from pancreas β-cells through certain glucose transporters. Then, glucose is changed to glucose-6-phostpahte by gluocokinase enzyme (GK) present in β-cells and that is how ATP is generated. This suggests that insulin secretion mostly depends on the glucose transport system, GK and glucose in β-cells [28].

Based on the search engine, the expression of genes related to cellular aging such as *p53*, *p38*, *p21*, and *p16* is significantly higher in aged islet cells in comparison to than young ones and this effect could be due to the increase of aged animals associated with the steep rise in the production of ROS. Similarly, low expression of *Glut2* gene in aged pancreatic islet cells creates a strong association between *Glut2* levels and the aging. Down-regulation of *Glut2* expression is more likely touched by the β-cell function decline as an index of cellular aging [29]. In aging, the alteration of insulin secretion parallel to *Glut2* up- or down-regulation appeared to be due to the many factors including influence on Sirt1-mediated GSIS [30], the sensitivity of β-cell to incretins [31], mitochondrial function [32] and most importantly oxidative stress [33]. The ROS production was reduced in islets cells as a result of

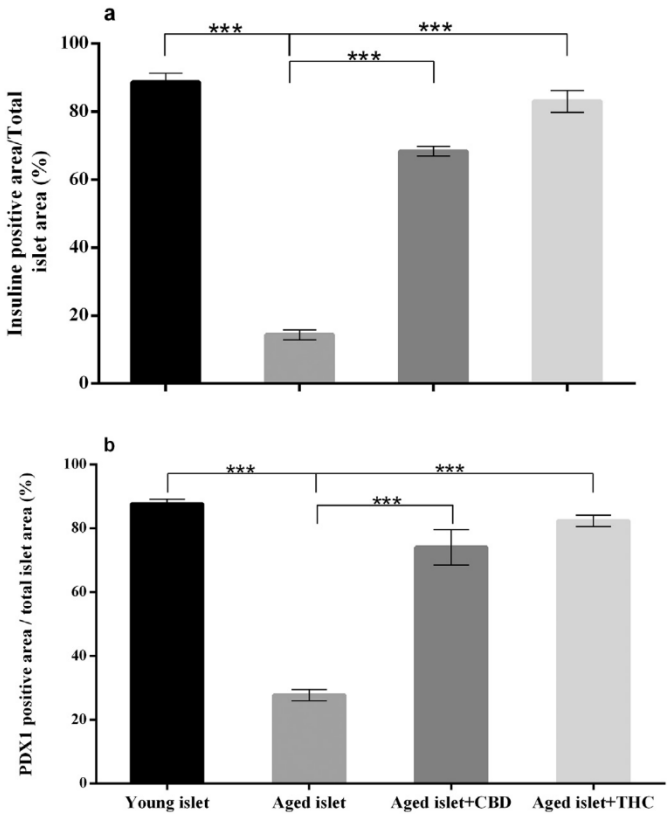

Fig. 4. The quantitative fluorescence intensity of insulin protein and Pdx1 (a, b). The values are expressed as mean ± SEM. ***, indicates significantly differences at $p < .001$.

islets treatment with cannabinoids, which is the mechanism remains to be elucidated [34].

In general, cell aging considerably induces oxidative stress in β-cells by generating ROS with *p38* and *p53* activates. Moreover, *p38* highest expression level was revealed by RT-PCR in the aged rat islet cells. It indicates that aging in β-cells can be initiated by the activation of *p38* and *p53* genes. In addition to the genes associated with cellular aging, the ROS production is assumed to be a pivotal feature to alter the phenotype of *p16*, *p21*, *p38*, and *p53* causing premature cell aging [35]. The findings of a pre-clinical study indicated that increased expression of *p38* is associated with premature aging in fibroblasts of conjunctivochalasis [36]. The protein expression of *p35* and *p21* as the coordinator of oxidative stress is linked with a high ROS production and, in turn, the senescence, helping to regulate both restrain and promote the cell aging [37,38]. Analogous to other studies, either *p16*, *p21*, *p38*, and *p53* genes or associated proteins are involved in the ROS production and, in turn, in cell aging [39]. In this regard, the antioxidants have the potential capability of reducing cell aging effects of *p53*, indicating that CBD and THC appeared to decrease the oxidative stress in β-cells.

It has been documented that specific cannabinoids show certain actions against the underlying mechanisms intricate in neurodegeneration including oxidative stress, inflammation and ecotoxicity. Concerning these targets, cannabinoids might persuade the opposite effects. Both CBD and THC activate cannabinoid receptors, but on the other hand, they also act as antagonists of those receptors. Hence, the impact of cannabinoids on cell viability in a dose-dependent manner as well as [40] the ratio of CBD and THC when utilized together can evade the psychoactive effects as reported by other investigators [41]. In a broad sense, it has been suggested that CBD and THC extensively suppress factors contributing to the aging process in islet cells. In support of our findings, β-cells showed an alteration in insulin secretion as compared to the control group. Also, double immunostaining assay of Pdx-1 in pancreatic islet cells showed that exposing aged rat islets cells to CBD and THC caused a considerable amount of Pdx1, which in turn leads to a significant increase in insulin level [42]. The antioxidant power of pancreas β-cells appeared not to be powerful enough and is very sensitive to toxic effects of metabolites and aging, making it more defenseless against ROS.

These results showed that the treatment of aged islet cell with CBD and THC elevates the expression of insulin and Pdx1 levels in aged islets. And that may ensure the healthy aging extension.

5. Conclusion

The study of the CBD and THC on reducing the cell aging process in endocrine pancreas islet cells proved to be a powerful tool to peruse the cell aging process in aged rats. These compounds have protective effects on aged rats' pancreas with the capability of reducing of the aging-related metabolic disorders by repressing the expressions of *p53*, *p38*, *p21*, and *p16* genes, lowering SA-β-gal activity as well as oxidative stress parameters, altering insulin secretion and pattern of Pdx-1 expression, and increasing levels of total thiol molecules. The exposure of islet cells to THC could change the *Glut2* expression as a critical glucose transporter involved in the aging of pancreatic β-cells. The obtained results are preliminary; more studies are needed to address the cellular and molecular mechanisms by considering more enzymes and biochemical aspects regarding glucose metabolism. CBD and THC need to be further evaluated in terms of clinical and pharmaceutical elements to explore their potential beneficial effects in the treatment of diabetes.

Acknowledgments

This work as a part of a project was supported by Tehran University of Medical Sciences [Grant number 97-01-45-37663 & 98-01-222-41117]. Iran National Science Foundation (INSF) is acknowledged for the Seat Award directed to the corresponding author.

Abbreviations

ROS	Reactive oxygen species
T2D	Type 2 diabetes
MAPK	Mitogen-activated kinase pathway
CDK1	Cyclin-dependent kinase 1
CBD	Cannabidiol
THC	Tetrahydrocannabinol
TTM	Total thiol molecules
GK	Gluocokinase enzyme
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
BSA	Bovine serum albumin
Glut2	Glucose transporter 2
GAPDH	Glyceraldehyde 3-Phosphate dehydrogenase
DCFH-DA	2′,7′-dichlorofluorescein diacetate
DCFH	2′,7′-dichlorodihydrofluorescein

Author contributions

Conception and design of the study: MA, MB, MR, SMD. Acquisition of data: NNH, FK, SAS, SMN, HHA, ZB. Analysis and/or interpretation of data: MB, MR, SMD, FR. Drafting the manuscript: MA, FR, MB, MR, SMD. Revision and approval of the submitted version: MA, FR, MB, MR, SMD, NNH, FK, SAS, SMN, HHA, ZB.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

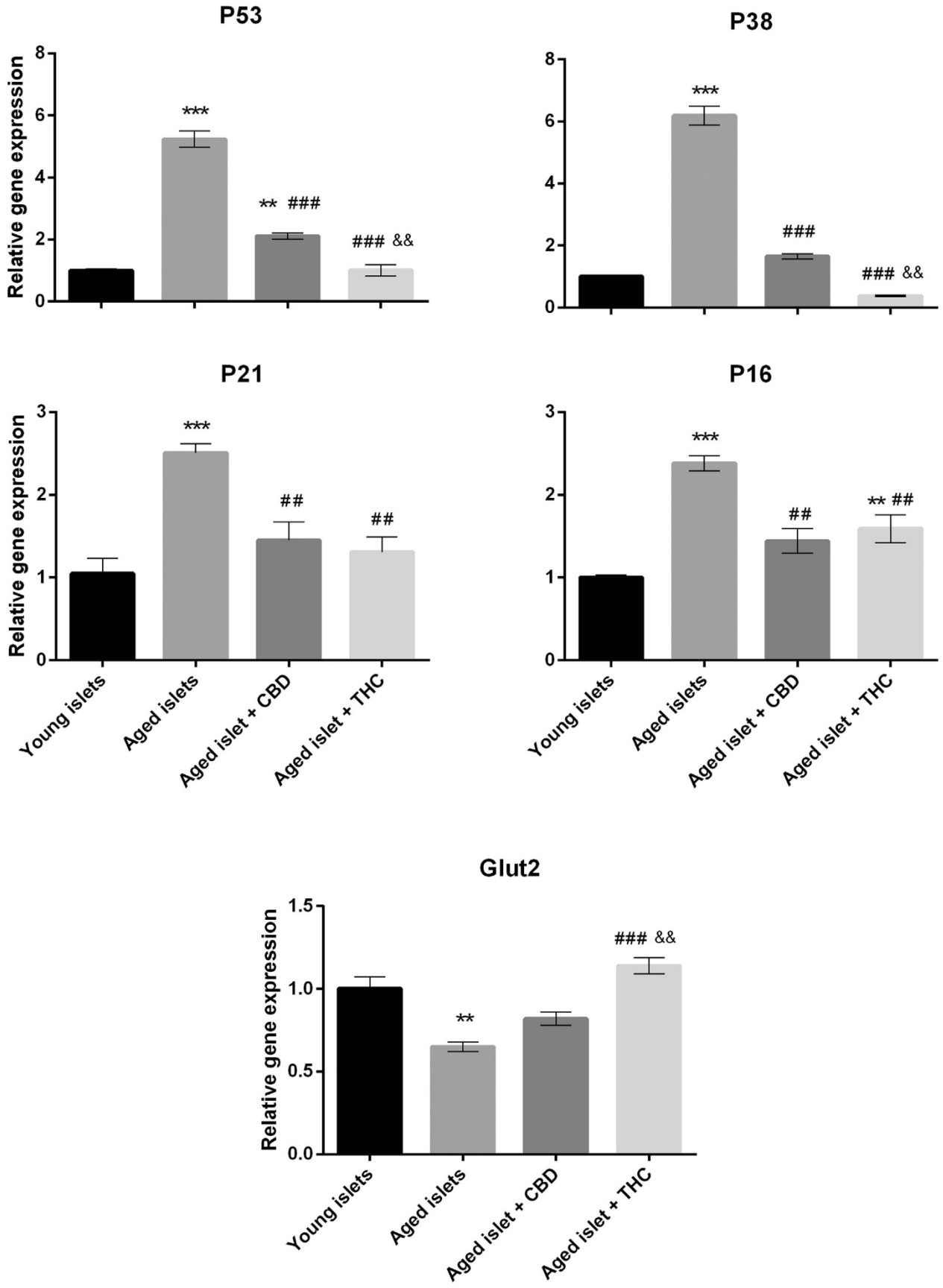

Fig. 5. Effects of ten μM of cannabidiol (CBD) and tetrahydrocannabinol (THC) on expression patterns of *p53*, *p38*, *p21*, *p16,* and *Glut2* genes in pancreatic islets from Wistar rats 24 h after treatment. The values are expressed as mean ± SEM of 4 groups of islets for each case, ($n = 10$). **, ***, significantly different from young islets group at $p < .01$ and $p < .001$, respectively. ##, ###, significantly different from aged islets group at $p < .01$ and $p < .001$, respectively. &&, significantly different from aged islets + CBD group at $p < .01$.

Table 2
Effect of ten μM cannabidiol (CBD) and tetrahydrocannabinol (THC) on β-galactosidase activity in pancreatic islets from aged Wistar rats 24 h after treatment. The values are expressed as mean ± SEM of 4 groups of islets for each case, (n = 10). **, significantly different from young islets group at $p < .01$. *, ***, significantly different from aged islets group at $p < .05$ and $p < .001$, respectively.

Group	Young islets	Aged islets	Aged islets + CBD	Aged islets + THC
β-galactosidase activity (mIU/ml)	3.75 ± 0.18	5.18 ± 0.26**	4.85 ± 0.24 ***	3.85 ± 0.19 *

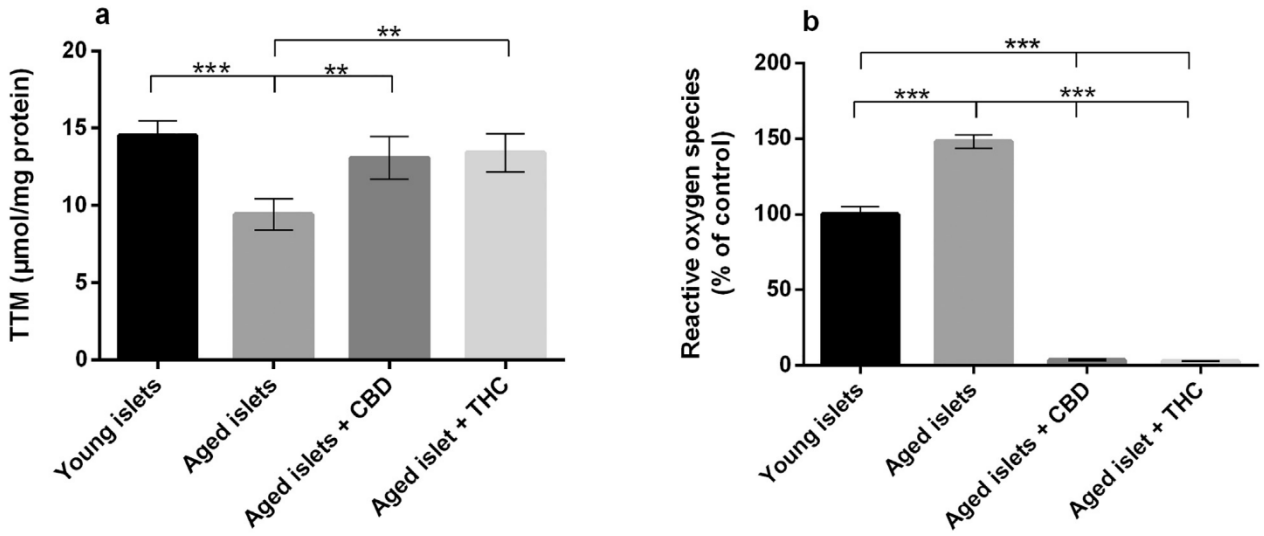

Fig. 6. Effects of ten μM cannabidiol (CBD) and tetrahydrocannabinol (THC) on oxidative stress in pancreatic islets from Wistar rats 24 h after treatment. TTM (total thiol molecules) (a) and ROS (reactive oxygen species) (b). The ROS values are presented as a percentage of the control group. The values are expressed as mean ± SEM of 4 groups of islets for each case, (n = 10). *** and ** indicate significant differences at $p < .001$ and $p < .01$, respectively.

Table 3
The analysis of correlation between *p53*, *p38*, *p21* and *p16* gene expressions as aging biomarkers, Pdx1, *Glut2* and insulin level functional markers, in rat pancreatic islets. * and **, indicate the significant correlation at 0.05 and 0.01 level respectively (2-tailed).

		Insulin	*p53*	*p38*	*p21*	*p16*	Pdx1	*Glut2*
Insulin	Pearson Correlation	1	−0.298	−0.376	−0.358	−0.403	0.369	−0.058
	Sig. (2-tailed)		0.702	0.624	0.642	0.597	0.631	0.942
p53	Pearson Correlation	−0.298	1	0.991**	0.985*	0.901	−0.994**	−0.906
	Sig. (2-tailed)	0.702		0.009	0.015	0.099	0.006	0.094
p38	Pearson Correlation	−0.376	0.991**	1	0.965*	0.863	−0.984*	−0.895
	Sig. (2-tailed)	0.624	0.009		0.035	0.137	0.016	0.105
p21	Pearson Correlation	−0.358	0.985*	0.965*	1	0.963*	−0.996**	−0.830
	Sig. (2-tailed)	0.642	0.015	0.035		0.037	0.004	0.170
p16	Pearson Correlation	−0.403	0.901	0.863	0.963*	1	−0.937	−0.668
	Sig. (2-tailed)	0.597	0.099	0.137	0.037		0.063	0.332
Pdx1	Pearson Correlation	0.369	−0.994**	−0.984*	−0.996**	−0.937	1	0.857
	Sig. (2-tailed)	0.631	0.006	0.016	0.004	0.063		0.143
Glut2	Pearson Correlation	−0.058	−0.906	−0.895	−0.830	−0.668	0.857	1
	Sig. (2-tailed)	0.942	0.094	0.105	0.170	0.332	0.143	

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