Vol.66: e23220138, 2023
https://doi.org/10.1590/1678-4324-2023220138
ISSN 1678-4324 Online Edition

Article - Human and Animal Health

The Potential Protective Role of Neoeriocitrin in a Streptozotocin-Induced Diabetic Model in INS-1E Pancreatic ß-Cells

Eymen Ece Aldemir[1]
https://orcid.org/0000-0002-5305-8797

Güldeniz Selmanoğlu[1]*
https://orcid.org/0000-0001-6565-736X

Elif Karacaoğlu[1]
https://orcid.org/0000-0003-3426-4584

[1]Hacettepe University, Faculty of Science, Department of Biology, Beytepe, Ankara, Turkey.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 23-Feb-2022; Accepted: 26-Jul-2022.

*Correspondence: guldeniz@hacettepe.edu.tr; Tel.: +90-312-2976433 (G.S.).

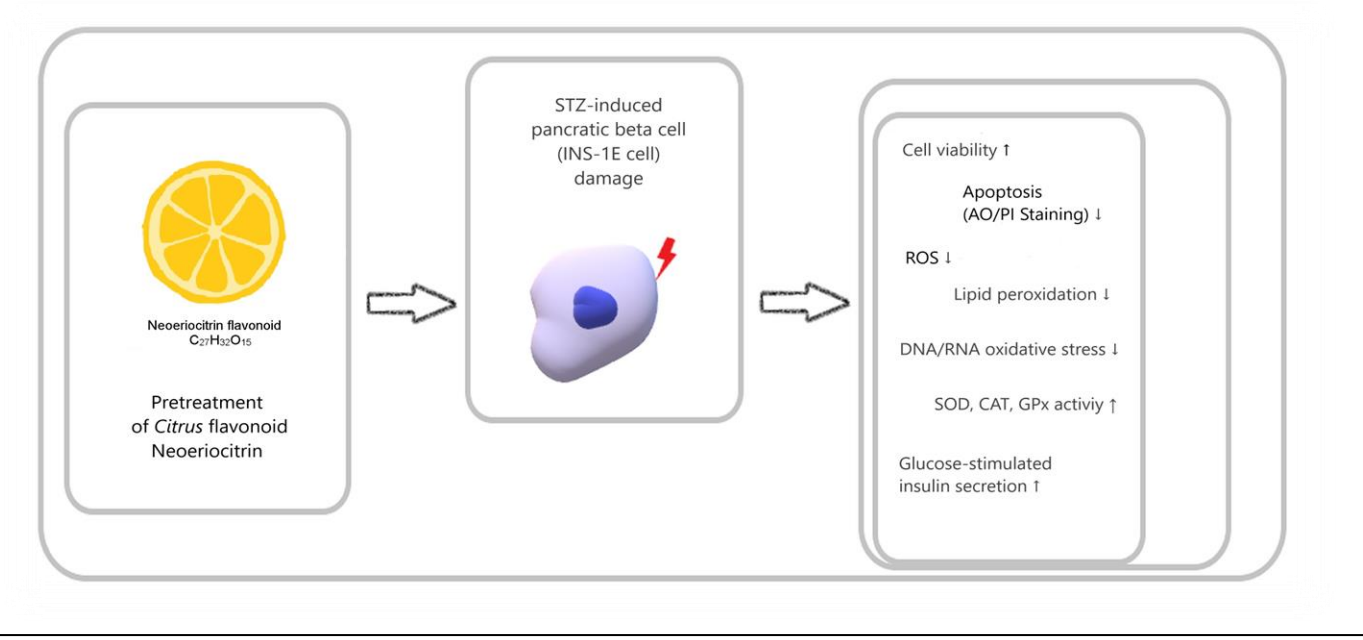
HIGHLIGHTS

- The potential protective effect of Neo in the STZ-induced damage in INS-1E cells was evaluated.
- The protective role of Neo on cell viability and reactive oxygen species formation were evaluated.
- Neo prevented ROS formation and elevated insulin levels in high glucose conditions.
- Pretreatment of Neo might be a candidate agent to prevent ROS in STZ-induced diabetes.

Abstract: Diabetes mellitus is a very common metabolic disorder. In addition to the use of drugs, plant-based antidiabetic products are also used as an alternative therapy. Although the main cause of the β-cell damage is not known exactly, it is attributed to oxidative stress. Although antioxidant effects of flavonoids are well known, there is an information gap about neoeriocitrin (Neo). Neo is found in fruit extracts, fruit juices of some plants. In this study, the potential protective effect of pretreatment of Neo was examined in pancreatic INS-1E cells against STZ-induced damage. INS-1E cells were pre-treated with Neo (0.25, 0.5, 1 µM) for 21 hours, and diabetes was induced by STZ. The cell viability, thiobarbituric acid reactive substances (TBARS) assay, DNA/RNA oxidative stress assay, and DCFHDA reactive oxygen species assay (ROS) were performed. Furthermore, the antioxidant enzyme activities and a glucose-stimulated insulin secretion assay (GSIS) were performed. Pretreatment of INS-1E cells with Neo caused increases in the cell viability, decreases in the apoptotic cell number in STZ-induced damage. Neo caused changes in GSIS levels, intracellular ROS level was decreased by neo pretreatment, it caused fold changes of lipid peroxidation, and DNA&RNA oxidative levels changed according to the STZ group. Additionally, pretreatment of Neo caused changes in fold changes of antioxidant activities in STZ-induced damage. Taking into account, pretreatment of neo has anti-oxidative effects, it is anticipated that neo might serve as a novel agent for the treatment of diabetes. However, further studies need to perform to enlighten the exact mechanism of action.

Keywords: Antioxidant; diabetes; INS-1E pancreatic beta-cell; neoeriocitrin flavonoid; oxidative stress.

GRAPHICAL ABSTRACT



INTRODUCTION

The main health problem of recent years is the remarkable increase in the incidences of metabolic diseases due to unhealthy and irregular diets globally. Diabetes is the most common metabolic disease in the world and it is estimated by 2030 diabetic adults will be 578 million [1]. According to the International Diabetes Federation, about 4 million people aged between 20-79 died due to diabetes-related issues in 2019 [1]. Diabetes mellitus is a complex metabolic disease and is mainly classified as type 1 and type 2. Diabetes can induce many diseases such as nephropathy, visual impairment, and neuropathy [2]. One of the reasons for the damage in pancreatic β-cells is the irregularity of insulin secretion, differences in response to glucose, and impaired glucose-insulin binding and insulin resistance [3]. High glucose in the blood triggers oxidative stress. Pancreatic ß-cells express GLUT-2 insulin carrier at a high glucose condition. Inadequate expression of antioxidant enzymes causes oxidative stress in the cell [4]. Oxidative stress causes damage to DNA or acts by the reduction of Ca+2 in the endoplasmic reticulum organelle of the pancreas ß-cells [5]. Oxidative stress has a critical function in pancreatic beta cell proliferation as well as cell survival physiologically or in pathophysiological situations. Furtherly, pancreatic β cells are known to be highly sensitive to oxidative stress conditions because of having low levels of antioxidant enzymes and they produce highly endogenous reactive oxygen species [6]. It is known that pancreatic ß cells undergo apoptosis as a result of glucotoxicity [7].

In modeling type 2 diabetes, several agents such as streptozotocin (STZ), hydrogen peroxide, and alloxan were used in in vitro and in vivo studies [8]. STZ is an alkylating agent by-product of Streptomyces achromogenes as a naturally occurring agent and cytotoxic analog of glucose [9]. STZ is known to cause ROS and nitric oxide formation. Also, STZ causes the breakdown of these molecules especially by affecting DNA, protein, and lipids, and leads to cell apoptosis [10]. It has been suggested that STZ treatment might successfully simulate the oxidative stress condition of diabetes in pancreatic cells [11].

The treatment of diabetic patients by conventional methods has long been known. Flavonoids are known to prevent oxidative stress due to their antioxidant properties. The use of alternative therapies, including functional foods and nutraceuticals, in cases where diabetes has a wide range of complications and medications, are insufficient and are among the recommended methods for controlling diabetes [12]. Neo (Eriodictyol 7-O-neohesperidoside) is one of the flavonoids that was recently found. Neo was extracted and isolated from the Citrus family such as satsuma pomegranate, Takumanatsukunenbo, and youth fruit in 1971 [13]. Li and colleagues investigated the effect of Neo on osteogenic differentiation [14]. Its structure is very similar to naringin flavonoid, which its antioxidant activity is known, and may also serve as a diabetes protective agent [6].

In this study, by considering the antioxidant and antidiabetic properties of flavonoids, we aimed to evaluate the antidiabetic and antioxidant properties of Neo flavonoid in INS-1E pancreatic β cells against

STZ-induced damage. In our study, the potential protective effect of Neo was examined by cell viability tests, oxidative stress parameters, cell death type, and insulin secretion tests.

MATERIAL AND METHODS

Chemicals and Reagents

Neoeriocitrin ($C_{27}H_{32}O_{15}$, Purity: 95%, CAS NO: 13241-32-2, Santa Cruz, Synonym: (S)-3′,4′,5,7-Tetrahydroxyflavanone-7-[2-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside]; Eriodictyol-7-neohesperidoside) was dissolved in dimethyl sulfoxide (DMSO). Streptozotocin (C8H15N3O7, Purity: ≥98%, CAS Number:18883-66-4, Santa Cruz, Synonym:2-Deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose) was dissolved citrate buffer solutions.

Culture of INS-1E cells and Establishment of the Experimental Groups

The INS-1E rat pancreatic ß cell line was supplied by Pierre Maechler and his colleagues (Geneva, Switzerland) [15]. The cells used in this study were approximately between 55 to 70 passages. INS-1E cells were cultured in 10% fetal bovine serum, 1 mM sodium pyruvate, 50 µM 2-mercaptoethanol, 2 mM L-glutamine, 1% penicillin/streptomycin, 10 mM HEPES in RPMI 1640 medium containing buffer, with 5 % CO_2 37 °C in the incubator.

INS-1E cells were treated with 1-5 mM STZ to induce an experimental diabetic model and IC_{50} was calculated. The preliminary study results revealed that 5 mM STZ induced cell damage approximately 40-50 % by crystal violet assay. For this reason, 5 mM STZ was found appropriate concentration for further analyses to induce cell damage and mimick diabetes model in vitro conditions. Neo was dissolved in DMSO for stock concentration. Concentrations used for further analyses were diluted by culture medium. Six experimental groups were as follows: Control group, 5 mM STZ, 0.25 µM Neo+STZ, 0.5 µM Neo+STZ, and 1 µM Neo+STZ groups and 1 µM NAC+STZ was used as a positive control group.

Determination of Cell Viability by Crystal Violet Assay

INS-1E cells were seeded in a 96-well plate at $2x10^5$ cells/ml density and incubated for 24 hours to adhere. The culture medium was renewed with/without flavonoids and cells were incubated for 21 hours. Then the cells in the experimental groups were incubated with 5 mM STZ except for the control group for 3 hours. Cells were fixed with 10 % formaldehyde for 45 min and incubated with 0.1 % crystal violet solution for 30 min. After removal of excess dye, crystal violet was extracted with 10 % acetic acid and optical density at 595 nm was recorded with a microplate reader (Bio-Tek µQuant, Bio-Tek Instruments, Inc., USA). The percentage of the cell viability of all groups was calculated according to the control group.

Determination of Cell Viability by Trypan Blue Assay

Trypan blue cell viability assay was also performed to evaluate the viable cells in response to treatment groups. Briefly, INS-1E cells were seeded into 6 well plates ($1x10^6$ cells/ml). Cells were incubated at 37 ºC, 5 % CO_2 for 24 hours to adhere the cells to the surface. After the treatment of the flavonoid and STZ, the suspended cells were withdrawn from each well and adhesive cells were detached from the surface with trypsin/EDTA. 100 µl trypan blue dye was added to 900 µl of suspended cells and the cells were counted on a Bürker's counting chamber under the light microscope. Live and dead cells were counted and the percentage of the cell viability was calculated according to the percentage of the cell viability was 100 % for the control group.

Acridine Orange-Propidium Iodide (AO/PI) Staining

Acridine orange (AO)/propidium iodide (PI) staining was one of the dual staining used in cell culture to detect cell death pathways. Apoptosis in the cell starts from the nucleus and secondary necrosis in cell membranes develops in later stages of the cell, although they are preserved. With this event, fragmentation occurs in the cell membrane (Gules and Eren, 2014). INS-1E cells were seeded at $2.5x10^5$ cells/ml in a 24-well plate. After Neo and STZ treatment, the cells were rinsed two times with PBS and stained with AO/PI (1 µg/ml:1 µg/ml) fluorescent dye. After washing with PBS, cell images were taken with an inverted microscope (Olympus IX70, Japan).

Glucose Stimulated Insulin Secretion (GSIS) Assay

INS-1E cells were seeded in 24-well plates ($2.5x10^5$ cells/ml) and incubated with flavonoids and STZ. To collect the cell lysate, the cells were left in the incubator for 2 hours in the glucose-free medium. After adding Hank's balance buffer (HBSS) containing 5.5 mM glucose into wells, the cells were incubated for 1 hour and the medium was collected. The cells were incubated with HBSS containing high glucose (16.7 mM) for 1 hour, then the lysates were collected and stored at -80 ºC. Insulin amount was measured with commercial insulin ELISA kit (SPI BIO, A11105, Bertin, France). The absorbance of lysates was measured in a microplate reader at 540 nm (Bio-Tek µQuant, Bio-Tek Instruments, Inc., USA). Data were expressed as ng/mg protein.

Measurement of Intracellular Reactive Oxygen Species (ROS)

The intracellular ROS production was determined by a 2',7'-Dicholorofluorescein diacetate (DCFHDA) method [16]. INS-1E pancreatic beta cells were seeded on a 96-well black plate ($5x10^4$ cells/well). After flavonoid and STZ treatment with a serum-free medium, the cells were rinsed with PBS. Hydrogen peroxide was used as a positive control for the DCFHDA assay. The DCFHDA (Sigma, D6883) dye was added to each well at 10 µM and left for 30 minutes to allow the dye to enter the cell and retain ROS. After washing of cells with PBS, the fluorescence intensity of cells was measured at 485-535 nm wavelength by fluorescence spectroscopy (EnSight ™ Multimode Plate Reader, Perkin Elmer).

Lipid Peroxidation Assay

INS-1E cells were cultured in 25 cm^2 filtered flasks. After treatment with flavonoids and STZ, cells were lysed with PBS, and the supernatants were collected. Lipid peroxidation assay was performed by using a commercial TBARS kit (Cayman, Germany, 10009055). Briefly, 100 µl of supernatants/standards and 100 µl of SDS solution were added into 5 ml vials and mixed well. After that, 4 ml of color reagent was added and vials were boiled for 1 hour. The samples were removed immediately and taken onto the ice to stop the reaction and incubated for about 10 min on ice. Then, samples were centrifuged at 1.600 g at 4ºC for 10 min. 150 µl of samples were transferred into 96 well plates and absorbances were measured at 530-540 nm with a microplate reader (Bio-Tek µQuant, Bio-Tek Instruments, Inc., USA).

DNA/RNA Oxidative Stress Measurement

Guanine base is the most sensitive to oxidation, and in process of the repairing cells, 8-hydroxyguanosine species are released from cells. In this context, in our study, we determined the three different oxidized guanosine derivatives. The DNA/RNA oxidative stress assay was performed by a commercial ELISA kit (Cayman, Cat no: 589320). After cells were treated by Neo followed by STZ, cell culture media was collected and an ELISA kit was performed according to directives of the kit manual. Finally, samples were measured in a microplate spectrophotometer at 405-420 nm wavelength.

Antioxidant Enzyme Activities

Antioxidant enzyme activities of SOD, CAT, and GPX were measured following treatment of Neo and STZ. After treatment of cells, cell lysates were prepared by scraping cells in lysis buffer on ice for 5 min. Samples were centrifuged at 15000 rpm at +4ºC, and supernatants were collected and stored at -80ºC. Enzyme activities were measured by commercial kits (SOD Assay kit, Cayman, # 706002, USA, CAT Assay kit, Cayman # 707002, GPX Assay kit, Biovision, # K762-100, USA). Enzyme activities were standardized to mg protein, and results were given as fold change according to control cells.

Statistical Analysis

Each experiment was performed in triplicate. Statistical analysis was performed using the IBM SPSS statistics 23. Data were expressed as the mean±standard error (SE) and statistical significance was assigned at $p \leq 0.05$ level. One-way analysis of variance (Oneway ANOVA) was performed when one variance changed. According to the variance analysis result, the Tukey test was applied for statistically significant p values.

RESULTS

Cell Viability Assays

Inhibitory concentration (IC_{50}) was calculated for STZ treatment to INS-1E cells for 3 hours. The cell viability of the INS-1E cells was determined as 56.77 % for IC50 for 5 mM STZ by crystal violet assay which was found statistically significant (Figure 1A). The citrate buffer which was non-toxic was used to dissolve STZ in the study. Since citrate buffer was not significantly different compared to the control group, it was not used for further analyses. The pretreatment with Neo at the concentration of 0.25 µM, 0.5 µM, and 1 µM for 21 h resulted in a significant ($p<0.0002$) increase in cell viability by crystal violet test. The comparison was performed between the control and DMSO control group because flavonoids were dissolved in DMSO. No significant difference was found between the control and DMSO groups, in other tests control group was taken into consideration. Considering the viability percentage of INS-1E cells, it was found that Neo has a protective effect of 78.82 % cell viability similar to NAC flavonoid which cell viability percentage was 79.51 %. Increasing concentrations of Neo caused rises in the viability rate of INS-1E cells proportionally (Figure 1B). Trypan blue stain results showed that Neo increased the cell viability in a concentration-dependent manner (Figure 1C). Interestingly, according to trypan blue staining results, it was observed that Neo had a higher percentage of viability compared to NAC. However, data was not found significantly significant.

Determination of the cell death type was also carried out by AO/PI staining (Figure 1D). Images of the AO/PI staining are seen in Figure 2. It was determined that cell viability in Neo treatment groups was elevated in a concentration-dependent manner compared to the STZ group (Figure 1D). Cell viability percentage decreased in the STZ treatment group significantly compared to the control group. Additionally, the percentage of cell viability in the 0.25 µM Neo+STZ treatment group showed statistically significant decreases compared to the control group, but not significantly different from STZ treatment group. Curiously, elevation in the percentage of cell viability in 0.5 and 1 µM Neo+STZ treatment groups were significantly different compared to the STZ treatment group. Neo treatment groups were found to have fewer apoptotic cells compared to the STZ treatment group. Cell viability in the 1 µM Neo group was found at 90 % which is similar to those of the 1 µM NAC treatment group.

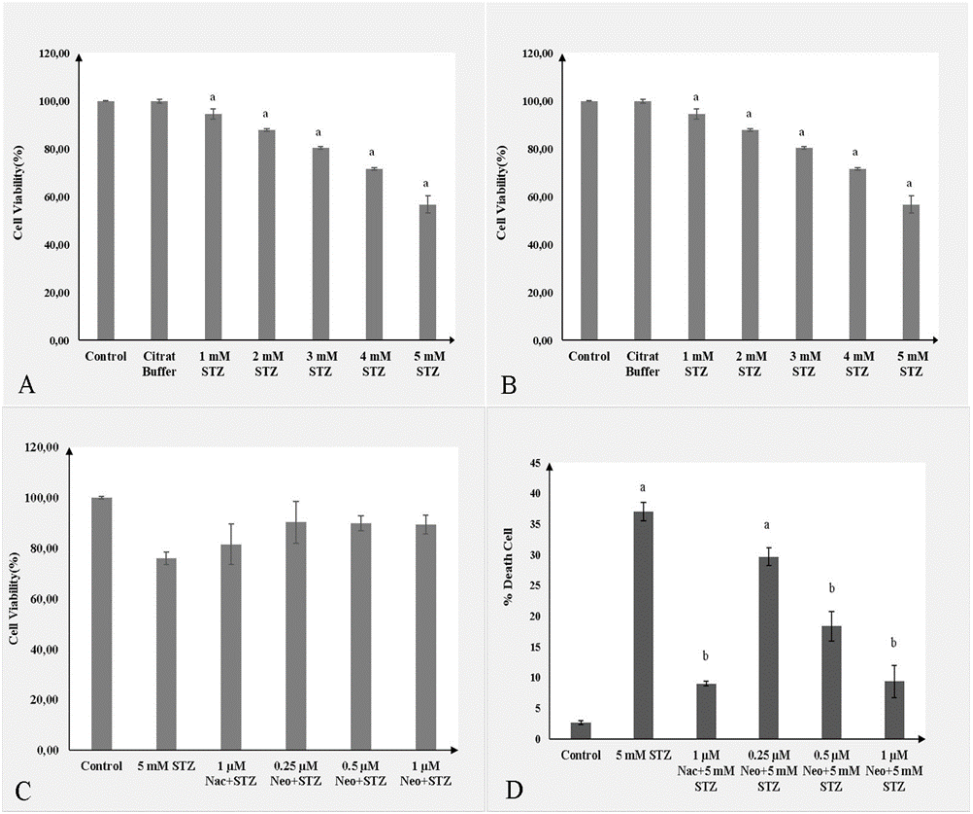


Figure 1. The percentages of the cell viability in INS1-E cells, A) result of crystal violet test in STZ treatment, B) Results of crystal violet test in the control and Neo+STZ treatment groups, C) Result of trypan blue test in control and Neo+STZ treatment groups, D) Result of acridine orange/propidium iodide test. [a]Significantly different from the control group ($p\leq0.05$). [b]Significantly different from the STZ group ($p\leq0.05$).

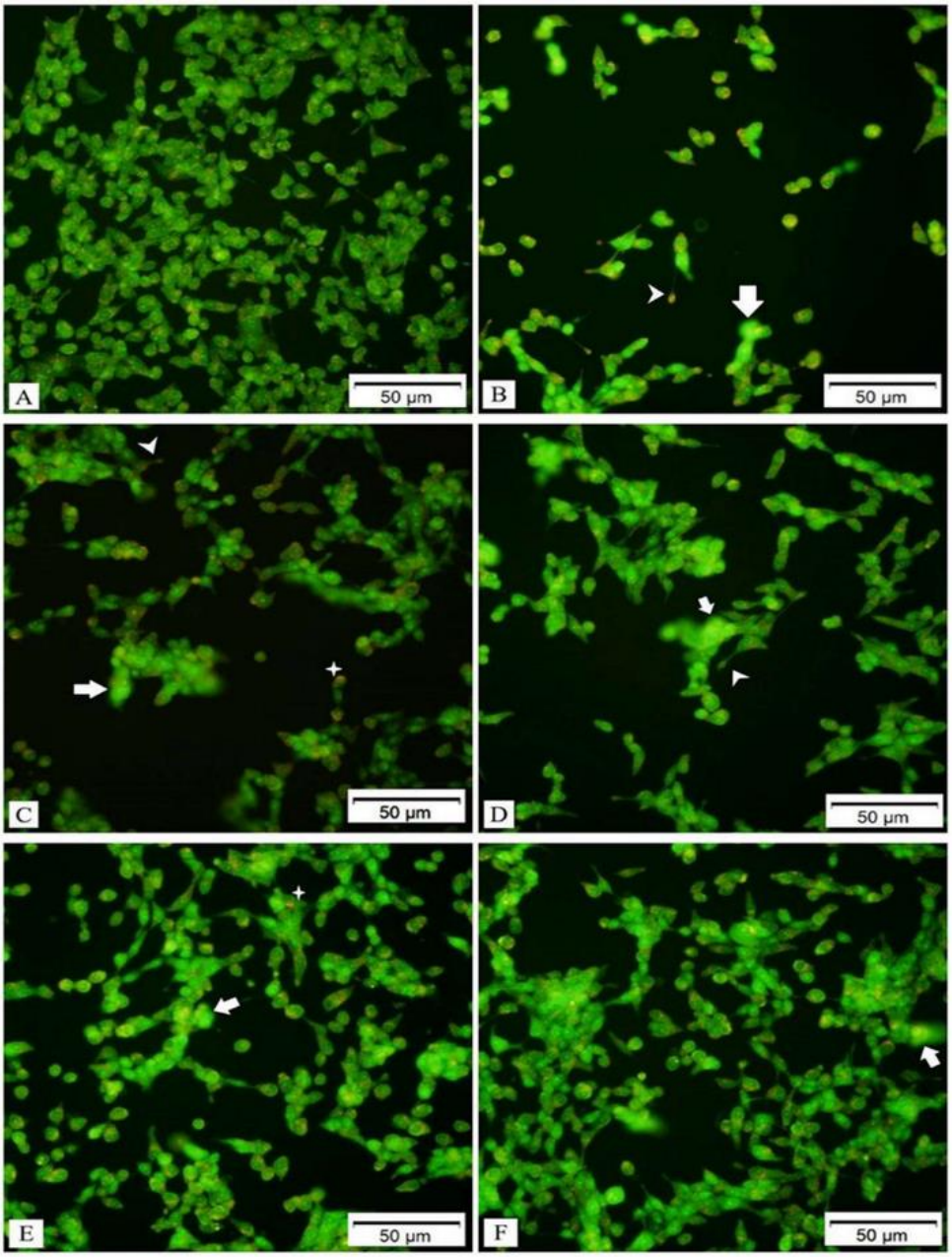


Figure 2. A) Control, B) STZ, C) NAC+STZ, D) 0.25 µM Neo+STZ, E) 0.5 µM Neo+STZ, F) 1 µM Neo+STZ. Apoptotic cells (→), early necrose (*), apoptotic bodies (►), and live cells were seen by fluorescence microscopy.

Glucose Stimulated Insulin Secretion (GSIS)

The insulin response of cells in the STZ treatment groups decreased in the low and high glucose conditions. While the decrease of insulin in low glucose conditions was not statistically significant, it was found statistically significant in high glucose conditions between the control and STZ treatment groups. Insulin response of INS-1E cells in low and high glucose medium at 0.5 µM and 1 µM concentrations of Neo elevated compared to the STZ treatment group (Figure 3). Insulin elevation in high glucose conditions was found statistically significantly compared to the STZ treatment group, however in low glucose conditions, elevation was not statistically significant for 0.5 µM Neo+STZ and 1 µM Neo+STZ groups.

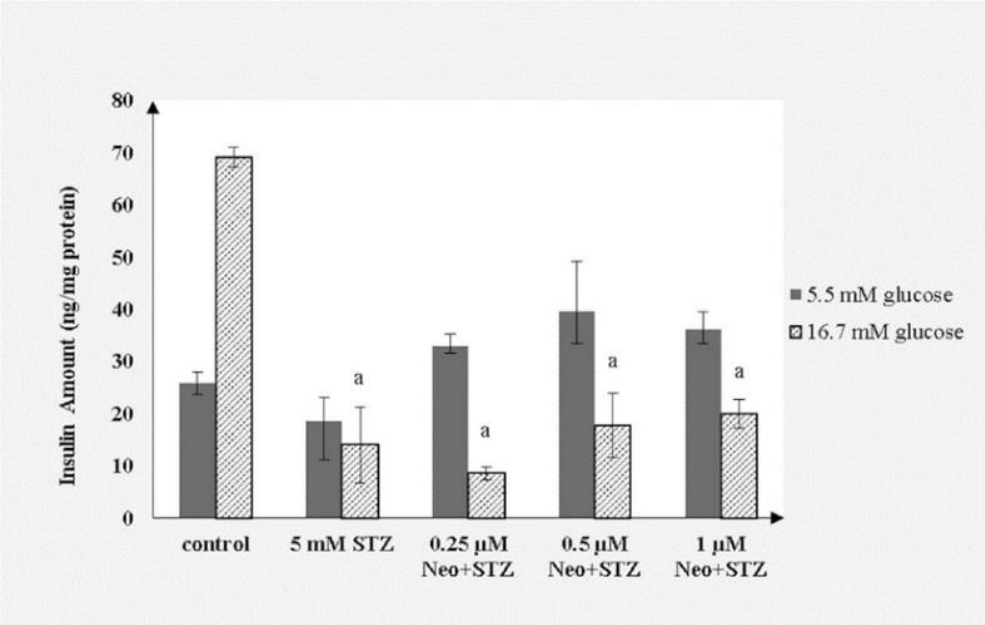


Figure 3. Effect of Neo on glucose-stimulated insulin secretion in INS-1E cells at low (5.5 mM) and high (16.7 mM) glucose concentrations. [a]Significantly different from the control group ($p \leq 0.05$). [b]Significantly different from STZ group ($p \leq 0.05$).

Measurement of Intracellular Reactive Oxygen Species (ROS)

ROS was determined by DCFHDA assay. Fold change of DCFDHA fluorescence intensity were shown in Figure 4A. DCFHDA assay showed increased ROS production in the STZ treatment group. There was a significant increase in fold changes in fluorescence intensity between the control and the STZ treatment group. The fluorescence fold change ratio decreased with increasing Neo concentration. It was observed that the hydrogen peroxide group has a statistically significant difference ($p \leq 0.05$) from other treatment groups, 1 µM Neo pre-treatment had less ROS formation than the control group (Figure 4A).

Lipid peroxidation was measured by TBARS assay spectrophotometrically. According to the lipid peroxidation assay, TBARS levels increased in the STZ treatment group, however, TBARS levels decreased with elevated neo concentrations. Although there was no statistically significant difference between groups, the cells in the STZ treatment group and 0.25 µM Neo+STZ treatment group were shown to produce more lipid peroxidation products than the control group. As seen in Figure 5, 0.5 µM and 1 µM Neo+STZ group was found to have lipid peroxidation product formation as much as control (Figure 4B). There is no statistically significant result between the groups.

DNA/RNA Oxidative Stress Assay Results

Results exerted that DNA/RNA oxidative stress increased in the STZ treatment group. Nevertheless, Neo treatment decreased the DNA/RNA oxidative stress but was not statistically significant. Results of the DNA/RNA oxidative stress were shown in Figure 4C.

The Antioxidant Enzyme Activity Results

The antioxidant enzyme activities in INS-1E cells following Neo and STZ treatment were measured. The graph of SOD activity fold change was given in Figure 5A. The SOD activity in the STZ treatment group increased compared to the control group, but the change was not found statistically significant. Additionally, the SOD activity fold change decreased in Neo treatment groups compared to the STZ treatment group, but it was also found not statistically significant.

The graph of the CAT activity fold change was shown in Figure 5B. According to assay results, the CAT activity fold change increased in response to STZ treatment, and it was also increased in the Neo treatment groups, but both changes were not found statistically different. Furthermore, the GPx activity fold change graph was shown in Figure 5C. The GPx activity fold change increased in response to Neo treatment. Especially, in the 0.25 µM Neo treatment group, but interestingly changes were not found statistically different.

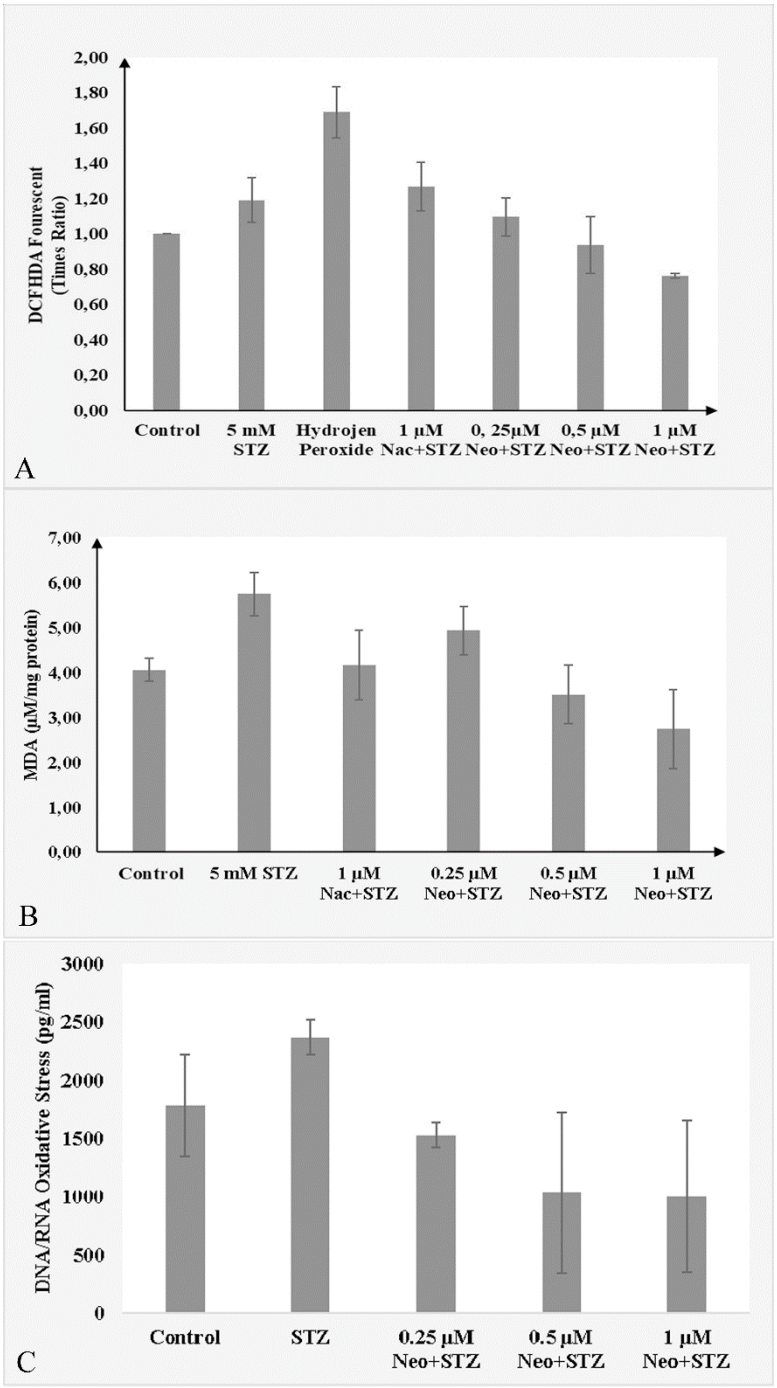


Figure 4. A) Intracellular ROS measurement fold change ratio in INS1-E cells in the control and the treatment groups, B) Lipid peroxidation measurement results of the control and the treatment groups in INS-1E cells, C) DNA/RNA oxidative damage measurement results of the control and the treatment groups in INS-1E cells.

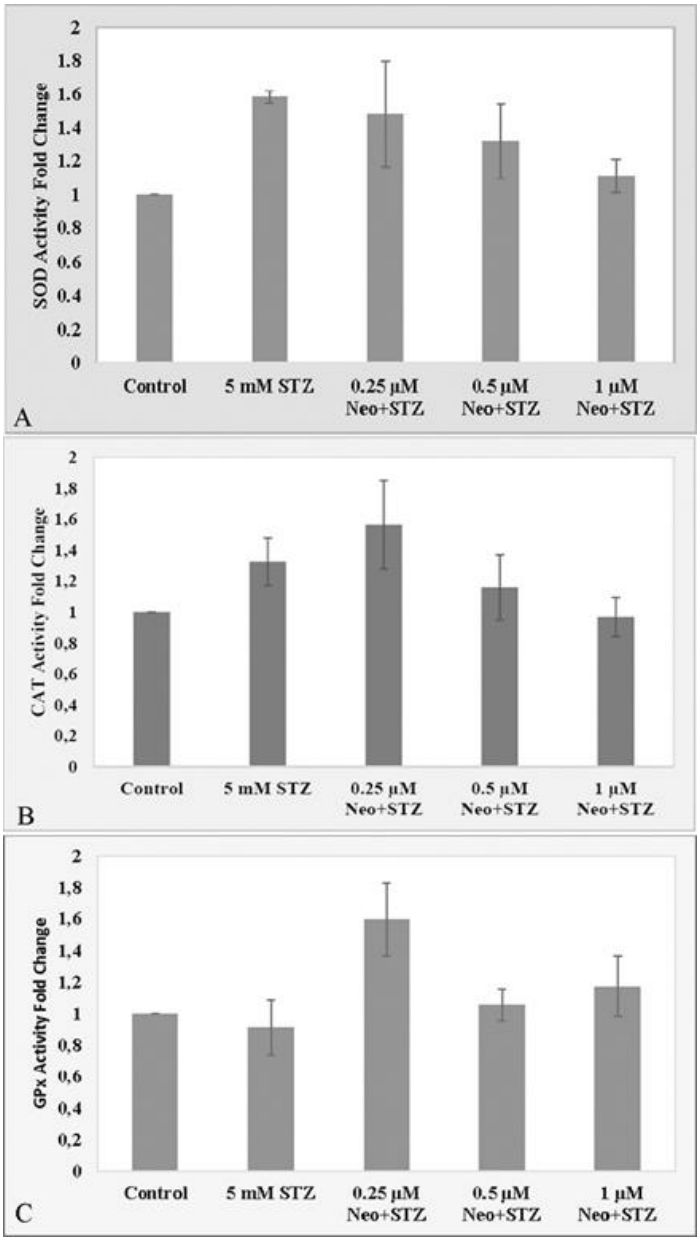


Figure 5. A) The SOD activity fold change results of the control and the treatment groups in INS-1E cells, B) The CAT activity fold change results of the control and the treatment groups in INS-1E cells, C) The GPx activity fold change results of the control and the treatment groups in INS-1E cells.

DISCUSSION

Diabetes is one of the most common metabolic diseases of our age. The number of people suffering from diabetes has increasing day by day. It is predicted that there will be 640 million diabetes patients in the world in 2040 [17]. Flavonoids belong to polyphenols with accounting 60 % of total phenolic substances. The most striking property of flavonoids is to exert antioxidant activity [18]. Previous studies revealed that oxidative stress plays a crucial role in both type-1 and type-2 varieties of diabetes. Permanent and high levels of ROS are known to impair the balance between oxidants and antioxidants, causing cell death by damaging proteins, lipids, and DNA [19]. Drugs used for diabetes focus on a single goal and fall short of analyzing complex pathology. Therefore, the use of non-pharmacological approaches, which may be potential alternatives in diseases affecting multiple signaling pathways could be promising [20]. -As a non-pharmacological approach, taking into account the antioxidant properties of flavonoids, in this study we aimed to evaluate the potential antioxidant and antidiabetic effects of Neo in STZ-induced cell damage in INS-1E pancreatic β cells.

A limited study about Neo flavonoids was reported. *Citrus bergamia* has significant amounts of various flavonoids such as naringin, neoeriocitrin, and neohesperidin [21]. Based on the antioxidant properties of *Citrus bergamia*, the antioxidant and antidiabetic effects of Neo were investigated. Pretreatment of Neo to

INS-1E pancreatic beta cells exerted high percentages of viable cells in the STZ-induced diabetes model by crystal violet assay. INS-1E cells showed preserved DNA structure by Neo pretreatment as live cells. Also, we revealed cell death type by STZ-induced damage and percentage of cell death by pretreatment of Neo by performing an AO/PI assay.

By examining the oxidative stress parameters, it was observed that Neo prevented pancreatic β-cell damage caused by intracellular ROS. In our study, we found that 1 µM Neo had a protective effect in STZ-induced INS-1E cells similar to the 1 µM NAC group. Our results showed a similar pattern to a study conducted with morin flavonoid. According to morin pretreatment, INS-1E cells had a higher percentage of cell viability against STZ treatment and caused a reduction in ROS formation [22].

The trypan blue is a negative charge stain that was used on cell culture and stains cells having membrane damage. In the present study, the trypan blue stain test result revealed an increase in cell viability depending on Neo concentrations. Our cell viability results were supported by AO/PI assay showing whether the cellular structures are disrupted. AO/PI fluorescence microscopy demonstrated that apoptotic bodies and apoptosis decreased in INS-1E cells treated with 1 µM Neo and 1µM NAC.

Glucose enters the cell through GLUT-2 channels, triggering insulin release. Especially, with high glucose uptake into the cell, insulin secretion increases. In this study, exposure of INS-1E cells to high glucose in STZ treatment groups resulted in a decrease in insulin secretion. However, pretreatment with 1 µM Neo is able to partially improve cell insulin secretion dysfunction. Despite this situation, 1 µM NAC pretreatment at low glucose concentration in INS-1E cells secreted more insulin than the control group. Taken together these findings suggested that decreased glucose secretion by STZ treatment in INS-1E cells, it seemed that Neo pretreatment could compensate for glucose secretion by slightly increase. In a study evaluating the effects of the dietary flavonoid quercetin in INS1 cells, it was reported that insulin secretion of cells increased in quercetin treatment at high concentration glucose conditions [23].

In a study that investigated the protective effect of epicatechin, a flavonoid found in cocoa, in INS-1E cells the amount of ROS was determined by DCFHDA and ROS amount decreased following epicatechin treatment [24]. STZ-induced ROS production decreased by Neo pretreatment depending on concentration. It can be asserted that Neo has a protective effect against oxidative stress by scavenging ROS.

Measurement of lipid peroxidation products was performed by TBARS assay. In the present study, a decrease in the amount of lipid peroxidation was detected according to the increasing concentration of Neo. The decrease in the amount of TBARS also supported that Neo had a protective effect against oxidative stress. Similar to our results, it was reported that the protective effect of octaphloretrol A against STZ-induced damage to RINm5F cells was evaluated, and pretreatment of octaphloretrol A resulted in reduced TBARS levels [25]. In the present study, TBARS amount decreased, in the same way, depending on the increase in Neo concentrations.

The endogenous antioxidant enzymes protect cells against reactive oxygen species. For this reason, the antioxidant enzyme activities were measured to evaluate the antioxidant capacity of Neo in INS-1E cells against STZ damage. In our study, we found that Neo has increased the CAT and the GPx activities slightly, but not SOD activity against the STZ-induced damage in INS-1E cells. Moreover, DNA/RNA oxidative damage was also decreased by Neo treatment in response to STZ damage in INS-1E cells. A previous study that evaluated the antidiabetic mechanism of the *Rosa canina* fruit was found to have protective effects on βTC6 pancreatic β-cell damage induced by STZ [26]. Similarly, RINm5F cell line damage was induced by STZ treatment, and apigenin was found to have protective effects against oxidative stress, as well as the antioxidant enzyme activities increased, and diminished DNA damage in response to apigenin treatment [27]. In another study, the phenolic compound Octalphlorethol A has been shown to reduce STZ-induced apoptosis and oxidative stress in RINm5F cells and increased insulin secretion. In the same study, it has been also reported that Octhaphlorethol A treatment increased the antioxidant enzyme activities, and diminished lipid peroxidation [25]. Our results were similar to those of other studies. When all the results were evaluated, it may be suggested that Neo had a certain amount of protective effect against STZ-induced oxidative damage.

CONCLUSION

Our study led us to conclude that Neo pretreatment to INS-1E pancreatic beta cells had protective effects against STZ-induced oxidative stress and cell damage. As a flavonoid, Neo may be a potential candidate agent against diabetes due to its antioxidant effect. This study could serve as preliminary data for further studies for elucidating the mechanisms of action of Neo on the prevention of diabetes with antioxidant activity.

Funding: This research was funded by Hacettepe University, Scientific Projects Coordination Unit (Project no: FBA-2018-16746).
Acknowledgments: Especially we would like to thank Dr. Maechler and colleagues for supplying the INS1-E cell line (Department of Cell Physiology and Metabolism University Medical Center, Geneva, Switzerland). A part of this research is a MSc thesis of Eymen Ece Aldemir.
Conflicts of Interest: Authors declare that there is no conflict of interest.

REFERENCES

1. Karuranga S, Malanda B, Saeedi P, Salpea P. International Diabetes Federation. 9th ed., Diabetes Atlas; 2019, 168 p.
2. Buchanan TA. Pancreatic beta-cell loss and preservation in type 2 diabetes. Clin. Ther. 2003; 25: B32-B46.
3. Bell GI, Polonsky KS. Diabetes mellitus and genetically programmed defects in β-cell function. Nature 2001, 414(6865): 788-91.
4. Kajimoto Y, Kaneto H. Role of oxidative stress in pancreatic β-cell dysfunction, in Mitochondrial Pathogenesis. Springer, Berlin, Heidelberg; 2004; pp 168-76.
5. Araki E, Oyadomari S, Mori M. Impact of endoplasmic reticulum stress pathway on pancreatic β-cells and diabetes mellitus. Exp. Biol. Med. 2003; 228(10): 1213-7.
6. Wang X-L, Wang N-L, Zhang Y, Gao H, Pang W-Y, Wong M-S, et al. Effects of eleven flavonoids from the osteoprotective fraction of Drynaria fortunei (Kunze) J. S M. on osteoblastic proliferation using an osteoblast-like cell line. Chemical and Pharmaceutical Bulletin 2008; 56(1): 46-51.
7. Chang-Chen KJ, Mullur R, Bernal-Mizrachi E. β-cell failure as a complication of diabetes. Rev. Endocr. Metab. Disord. 2008; 9(4): 329-43.
8. Etuk, E., Animals models for studying diabetes mellitus. Agr. Biol. J. N. Am. 2010; 1(2): 130-4.
9. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J. Diabetes Metab. Disord. 2013; 12(60): 1-7.
10. Takasu N, Komiya I, Asawa T, Nagasawa Y, Yamada T. Streptozocin-and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets: H_2O_2 as mediator for DNA fragmentation. Diabetes 1991; 40(9): 1141-5.
11. Wu J., Yan L-Y. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes Metab. Syndr. Obes. 2015; 8: 181-8.
12. Sun C., Zhao C, Guven EC, Paoli P, Simal-Gandara J, Mohanram K et al. Dietary polyphenols as antidiabetic agents: Advances and opportunities. Food Frontiers 2020; 1(1): 18-44.
13. Masao N, Shintaro K, Schiko E, Fumiko I. Flavonoids in Citrus and related genera Part II. Isolation and identification of isonaringin and neoeriocitrin from Citrus. Agric. Biol. Chem. 1971; 35(11): 1683-90.
14. Li L, Zeng Z, Cai G. Comparison of neoeriocitrin and naringin on proliferation and osteogenic differentiation in MC3T3-E1. Phytomedicine 2011; 18(11): 985-9.
15. Merglen A, Theander S, Rubi B, Chaffard G, Wollheim CB, Maechler P. Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells. Endocrinology 2004; 145(2): 667-78.
16. Iancu DS, Savopol T, Iancu CB, Kovacs E. Microvolumetric detection of reactive oxygen species in living cells. Rom. J. Biophys. 2012; 22: 31-9.
17. WHO, Global report on diabetes, WHO Library Catalogiung-in-Publication Data. 2016, Geneva: WHO Press. ISBN: 9789241565257.
18. Shahidi F, Varatharajan V, Oh WY, Peng H. Phenolic compounds in agri-food by-products, their bioavailability and health effects. J. food Bioact. 2019; 5(1): 57-119.
19. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ. J. 2012; 5(1): 9-19.
20. Tan SY, Wong JLM, Sim YJ, Wong SS, Elhassan SAME, Tan SH, et al. Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. Diabetes. Metab. Syndr. 2019; 13(1): 364-372.
21. Gabriele M, Frassinetti S, Caltavuturo L, Montero L, Dinelli G, Longo V, et al. Citrus bergamia powder: Antioxidant, antimicrobial and anti-inflammatory properties. J. Funct. Foods 2017; 31: 255-265.
22. Vanitha P, Senthilkumar S, Dornadula S, Anandhakumar S, Rajaguru P, Ramkumar KM. Morin activates the Nrf2-ARE pathway and reduces oxidative stress-induced DNA damage in pancreatic beta cells. Eur J Pharmacol. 2017; 801: 9-18.

23. Kittl M, Beyreis M, Tumurhtuya M, Fürst J, Helm K, Pitschmann A, et al. Quercetin stimulates insulin secretion and reduces the viability of rat INS-1 beta-cells. Cell. Physiol. Biochem. 2016; 39(1): 278-93.
24. Martín MÁ,Fernández-Millán E, Ramos S, Bravo L, Goya L. Cocoa flavonoid epicatechin protects pancreatic beta cell viability and function against oxidative stress. Mol. Nutr. Food Res. 2014; 58(3): 447-56.
25. Lee S-H, Kang S-M, Ko S-C, Kang M-C, Jeon Y-J. Octaphlorethol A, a novel phenolic compound isolated from Ishige foliacea, protects against streptozotocin-induced pancreatic β cell damage by reducing oxidative stress and apoptosis. Food Chem. Toxicol. 2013; 59: 643-9.
26. Fattahi A, Niyazi F, Shahbazi B, Farzaei MH, Bahrami G. Antidiabetic mechanisms of Rosa canina fruits: an in vitro evaluation. Evid. Based Complementary Altern. Med. 2017; 22(1): 127-33.
27. Wang N, Yi WJ, Tan L, Zhang JH, Xu J, Chen Y, et al. Apigenin attenuates streptozotocin-induced pancreatic β cell damage by its protective effects on cellular antioxidant defense. In Vitro Cell. Dev. Biol. Anim. 2017; 53(6): 554-63.