

Oligonol Attenuates Diabetes-Induced Pancreatic Damage by Inhibiting Inflammatory Responses via Oxidative Stress-Dependent MAPK/NF- κ B Signaling

**Chan Hum Park¹, Joo Young Lee², Sung Ho Shin³, Min Yeong Kim³, Eun Suk Lee¹,
Yu Su Shin¹, Young Ock Kim¹, Sang Won Lee¹, Jae Sue Choi⁴ and Takako Yokozawa^{5,*}**

¹ Department of Medicinal Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumseong, Korea;
ptman123@korea.kr (C.H.P.); chestnut7@korea.kr (E.S.L.); totoro69@korea.kr (Y.S.S.); kyo9128@korea.kr (Y.O.K.); swlee1004@korea.kr (S.W.L.)

² College of Korean Medicine, Daegu Haany University, Daegu, Korea;
jyhappy0130@hanmail.net

³ Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon City, Gyeongsanbuk-do 39660, Korea; ikarus121@hanmail.net (S.H.S.); lksmhyk69@naver.com (M.Y.K.)

⁴ Department of Food and Life Science, Pukyong National University, Busan 608-737, Korea; choijs@pknu.ac.kr

⁵ Graduate School of Science and Engineering for Research, University of Toyama, Toyama 2630, Japan

* Correspondence: yokozawa@inm.u-toyama.ac.jp; Tel.: +81-76-445-6667

Abstract: Oligonol is a low-molecular-weight polyphenol derived from lychee fruit. This study was conducted to examine whether oligonol has an ameliorative effect on diabetes-induced pancreatic damage via oxidative stress-induced inflammation. Oligonol was orally administered at 10 or 20 mg/kg body weight/day for 10 days to streptozotocin-induced diabetic rats, and changes in serum glucose, C-peptide, insulin, reactive oxygen species (ROS), and thiobarbituric acid-reactive substance (TBARS) levels as well as body weight and food and water consumption were assessed. Furthermore, rat pancreases were analyzed for weight, ROS generation, TBARS level, insulin content, and protein expressions of phosphor (p)-p38, p-extracellular-signal regulated kinase 1/2, p-inhibitor of nuclear factor kappa B α , nuclear factor-kappa Bp65, cyclooxygenase-2, inducible nitric oxide synthase, tumor necrosis factor- α , and interleukin-6. Markers of diabetes were shown to be decreased by oligonol administration and histological damage in the pancreas was also ameliorated. These results indicate that oligonol exerts antidiabetic activities, which may be mediated via antioxidative, stress-related, anti-inflammatory signaling.

Keywords: oligonol; diabetes; pancreas; antioxidative stress; anti-inflammation

1. Introduction

Glucose is the primary fuel and regulator of pancreatic islet β -cell function. The primary function of insulin is to maintain blood glucose levels in the normal range. However, this homeostatic relationship is disrupted when glucose remains at supraphysiologic levels for protracted periods of time, an event referred to as glucose toxicity [1-3]. Diabetes, one of the most recognizable endocrine metabolic disorders, is fundamentally characterized by hyperglycemia. Hyperglycemia is a well-distinguished pathogenic factor of chronic complications in diabetes, which results in generation of excessive free radicals (reactive oxygen species [ROS]) and attenuation of antioxidative defense mechanisms through glycation of antioxidant enzymes [4].

Herbal medicinal plants are of importance to the health of an individual as well as for communities. The beneficial effects of medicinal plants are typically attributable to the presence of several chemically active compounds that exert specific physiological activities in the human body. Among these, bioactive antioxidants have been used for the treatment of various organ dysfunctions, and fruits, vegetables, tea, and wine are the major sources of flavonoids or polyphenolic compounds known to have a strong antioxidant activity [5].

Lychee has been consumed since ancient times in China and the southern area of Southeast Asia. It is rich in polyphenols, with Brat et al. [6] reporting that its polyphenol content per edible part is second only to that of strawberries. A particular characteristic of lychee polyphenols is a phenolic product containing catechin-type monomers and oligomers of proanthocyanidins [7]. Proanthocyanidins are structurally characterized as polymers of catechin and have a high molecular weight. However, their absorption in the body is low when administered orally, resulting in relatively low in vivo activity. Moreover, proanthocyanidins with a high molecular weight are virtually insoluble in water and have an astringent taste, binding to salivary proteins and mucous membranes in the mouth [8] and rendering them difficult to use in the food industry. Tanaka et al.

[9] successfully converted the high-molecular-weight proanthocyanidin into the low-molecular-weight proanthocyanidin, which is utilizable in the food industry. Oligonol, which is now available commercially as a novel dietary ingredient, is an optimized phenolic product derived from lychee fruit polyphenols that contain catechin-type monomers and low-molecular-weight oligomers [7]. Kundu et al. [10] reported the beneficial antioxidant and anti-inflammatory effects of oligonol in mouse skin, while Ahn et al. [11] showed that oligonol protects against oxidative stress-induced inflammation in C6 glial cells. In our previous studies, oligonol improved hyperglycemia and hyperglycemia-induced oxidative stress in the liver and kidney of type 2 diabetic *db/db* mice, attenuated renal damage through the inhibition of glycation end product formation by glucose accumulation, and subsequently decreased NAD(P)H oxidase-derived ROS production in the kidney of *db/db* mice [12-14]. Therefore, oligonol appears to represent a novel therapeutic approach to a range of conditions associated with diabetes and hyperglycemia-related disorders such as oxidative stress and inflammation; however, the details of the mechanisms are still unknown.

In the present study, we therefore investigated the mechanisms underlying the protective action of oligonol in pancreatic oxidative damage caused by streptozotocin (STZ)-induced diabetes in a rat model.

2. Results

2.1. Body Weight, Food Intake, Water Intake, and Pancreatic Weight

During the 10 days of the experimental period, diabetic control rats showed a significantly lower body weight gain than the nondiabetic rats did, and the oral administration of 20 mg/kg oligonol to diabetic rats showed a tendency to increase body weight. The diabetic control rats

showed an increased food and water intake compared with that reported for the nondiabetic rats. There was, however, no significant reduction in these parameters following the administration of oligonol. The pancreatic weight of diabetic control rats was 1.91 times higher than that of nondiabetic rats but decreased significantly in the rats administered oligonol, as shown in Figure 1.

2.2. Hematological Analyses

As shown in Figure 2, diabetic control rats maintained a level of about 410 mg/dL, while nondiabetic rats showed a level of around 130 mg/dL; however, oligonol-administered groups showed a dose-dependent decrease in this level. Furthermore, diabetic control rats showed a marked decrease in serum C-peptide and insulin levels, but oral administration of oligonol at 10 or 20 mg/kg suppressed these decreased levels. The serum levels of ROS and TBARS in diabetic rats orally administered 10 or 20 mg/kg body weight of oligonol decreased in a dose-dependent manner (Table 1).

2.3. Pancreatic Insulin Content

As shown in Figure 3, the pancreatic insulin content of vehicle-treated diabetic rats significantly decreased compared with that of nondiabetic rats. Oligonol administration at doses of 10 and 20 mg/kg led to a significant increase in the level of insulin.

2.4. Biomarkers Associated with Oxidative Stress in the Pancreas

The pancreatic levels of ROS and TBARS in diabetic rats were higher than those of nondiabetic rats, whereas these enhanced levels were significantly reduced by oligonol treatment,

almost to the levels of nondiabetic control rats, as shown in Table 1.

2.5. Pancreatic p-p38 and p-ERK1/2 Protein Expressions

In the diabetic control rats, pancreatic p-p38 and p-ERK1/2 protein expressions were significantly higher than those in nondiabetic rats (Figure 4). However, oligonol administration reduced pancreatic p-p38 and p-ERK expressions in diabetic rats.

2.6. Pancreatic p-I κ B α and NF- κ Bp65 Protein Expressions

As shown in Figure 5, pancreatic p-I κ B α and NF- κ Bp65 protein expressions were significantly elevated in diabetic control rats compared with those in nondiabetic rats. These increases in protein expressions in the pancreas of diabetic rats were significantly attenuated in a dose-dependent manner by oligonol administration.

2.7. Pancreatic Inflammatory Protein Expressions

The pancreatic expressions of inflammatory proteins COX-2, iNOS, TNF- α , and IL-6 were enhanced in diabetic control rats; however, these increases in expressions were significantly attenuated following oligonol administration (Figure 6).

2.8. Pancreatic Histological Examination

Figure 7 shows the results of histological examination using HE staining to detect pancreatic cell damage. The level of cellular damage was higher in the pancreas of diabetic control rats than in

those of nondiabetic rats. These histopathological studies demonstrated that the administration of oligonol had a significant, protective effect against pancreatic cell damage.

3. Discussion

Oxidative stress secondary to hyperglycemia is thought to be a major risk factor in the onset and progression of diabetes. Moreover, insulin resistance and β -cell dysfunction may play an important role in the unbalanced redox equation [15]. The antioxidant defense system consists of endogenous antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase, and exogenous antioxidants obtained mainly by dietary intake such as vitamin C, vitamin E, carotenoids, and polyphenols [16-19]. Various polyphenols show antioxidant activity because of their free radical scavenging properties and because they are chelators of metal ions; thus, they may protect tissues against free oxygen radicals and lipid peroxidation [20].

The beneficial effects of oligonol in diabetes have been reported; however, its underlying mechanism has not been elucidated thus far. In this study, to examine the regulatory mechanism of oxidative stress and the oxidative stress-associated inflammatory response of oligonol in hyperglycemia, we focused on the effects of oligonol against pancreatic damage and insulin secretion linked to inflammation using an STZ-induced diabetic rat model.

Our results show that oligonol administration significantly ameliorates adverse metabolic effects in STZ-induced diabetic rats. Significant body weight loss was observed in STZ-induced diabetic rats in comparison with that in nondiabetic rats, whereas body weight following the oral administration of 20 mg oligonol was greater than in diabetic control rats. These results suggest that oligonol may normalize energy metabolism in body tissues. The stimulation of glucose utilization by insulin is one of the major mechanisms for regulation of serum glucose concentrations. Administering oligonol to diabetic rats significantly improved insulin levels and serum C-peptide,

an indirect biomarker of insulin secretion. Therefore, the capability of oligonol to significantly reduce serum glucose levels in diabetic rats may be due to the potentiation of insulin secretion from existing β -cell islets, which subsequently increases the utilization of glucose in tissue.

In the diabetic state, potentially damaging oxidative stress is induced in islets as well as in other cells [21-23]. Because the expression levels of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are known to be very low in islets compared with that in other tissues [24], β -cells could be particularly susceptible to oxidative stress. Several studies have provided evidence that oxidative stress caused by diabetes mediates the toxic effects of hyperglycemia on β -cells [25-29], suppressing insulin gene transcription and glucose-stimulated insulin secretion and even causing apoptosis in β -cells. Furthermore, some toxic effects of hyperglycemia on β -cells in rodent models were reported to be ameliorated by an antidiabetic treatment [26,29]. In the present study, oligonol administration suppressed serum and pancreatic ROS and lipid peroxidation, indicating that oligonol treatment protects against oxidative insults in the serum and pancreas of STZ-induced diabetic rats. These results further support our previous study on proanthocyanidins, especially oligomers, from persimmon peel [30], showing that antioxidative activity is an important mechanism in protection against diabetes. Hyperglycemia results in the generation of excessive ROS. The accumulation of ROS activates NF- κ B via the ERK1/2 and p38 mitogen-activated protein kinase (MAPK) pathways, resulting in the expression of proteins encoding inflammatory molecules [31]. Based on the aforementioned data, we propose that the hypoglycemic potential of oligonol may be attributed, at least partly, to a reduction in ROS with subsequent reduction of lipid peroxidation, thus preserving insulin secretion by reducing β -cell death in the diabetic rat pancreas. Moreover, increased expression of NF- κ B by ROS production was also observed in the pancreatic tissue of vehicle-treated diabetic rats and was decreased by the administration of oligonol.

NF- κ B is a major proinflammatory transcription factor that plays key roles in the regulation

of transcription and expression of several genes implicated in a wide range of proinflammatory activities, including cytokines (TNF- α and IL-6) and enzymes (COX-2 and iNOS) [32]. The subsequent formation of NO and prostaglandin E₂ by hyperglycemia-induced iNOS and COX-2 expression, respectively, may impair pancreatic β -cell function [33]. However, the development of insulin resistance has been associated with NF- κ B and its target proinflammatory cytokines, such as TNF- α , IL-1, and IL-6 [34]. Furthermore, some studies have shown that TNF- α and IL-6 are positively correlated with measures of insulin resistance and/or plasma insulin concentration [35,36]. Therefore, NF- κ B and its target genes might be involved in the regulation of insulin secretion and insulin resistance. In addition, NF- κ B activation pathways are thought to be mediated by two distinct redox-related mechanisms: 1) phosphorylation of p38 MAPK, ERK, and c-Jun-NH₂-terminal kinase; and 2) enhancement of I κ B kinase-dependent I κ B degradation [37,38]. In the present study, the administration of oligonol to diabetic rats significantly reduced the renal protein expressions of NF- κ B and NF- κ B-related proinflammatory mediators (COX-2 and iNOS) and cytokines (TNF- α and IL-6). Oligonol was found to block this ROS-dependent activation of the MAPK pathway in the pancreas of diabetic rats. Moreover, oligonol administration resolved the p-I κ B levels elevated in the diabetes model, and also decreased the nuclear translocation of NF- κ B and NF- κ B-related protein expressions. These results suggest that oligonol can modulate diabetic pancreatic inflammation by regulating the MAPK-mediated NF- κ B signaling pathway. Furthermore, oligonol led to a reduction in pancreatic tissue inflammation based on histological evaluation.

This study focused on the effects of insulin secretion linked to inflammation in the pancreas of diabetic rats treated with oligonol. Our findings show that the administration of oligonol to diabetic rats had a favorable influence on the prevention of pancreatic damage progression, at least in part, by ameliorating hyperglycemia-related MAPK/NF- κ B pathway-induced inflammation. Given that oligonol inhibited oxidative stress-induced MAPK/NF- κ B activation-related inflammatory gene expression in the pancreas, these findings provide therapeutic evidence for the

beneficial effects of oligonol on ameliorating the development of diabetes-related pancreatic damage.

4. Materials and Methods

4.1. Oligonol

Oligonol obtained from lychee fruit (*Litchi chinensis* Sonn.) using a patented process (international patent WO 2004/103988 AI) [39] was provided by Amino Up Chemical, Co., Ltd (Sapporo, Japan). The composition of the product is shown in Table 2. Briefly, the extraction process included the combination of lychee fruit extract (750 g) and green tea extract (150 g) (purchased from Guilin Layn Natural Ingredients, Corp., Guilin, China) with citric acid (150 g) in water (7.5 L). The mixture was heated at 60°C for 16 h. After the solution was cooled to room temperature, it was filtered through a DIAION HP-20 column. The column was washed with water and eluted with 40% (vol/vol) ethanol (80 L). Removal of the solvent from the eluate yielded a reddish-brown powder (674 g) [40].

4.2. Materials

Protease inhibitor mixture solution, 4,6-dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid), and 10% neutral-buffered formalin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2',7'-Dichlorofluorescein diacetate (DCFH-DA) was purchased from Molecular Probes (Eugene, OR, USA). Pure nitrocellulose membrane was purchased from Bio-Rad Laboratories (Tokyo, Japan). Phenylmethylsulfonyl fluoride (PMSF) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rabbit polyclonal antibodies against insulin, phosphor (p)-p38,

p-extracellular-signal regulated kinase 1/2 (p-ERK1/2), p-inhibitor of nuclear factor-kappa B α (p-I κ B α) and nuclear factor-kappa B (NF- κ B)p65, and mouse monoclonal antibodies against cyclooxygenase-2 (COX-2), inducible nitric oxide (NO) synthase (iNOS), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), β -actin, and histone were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Goat anti-rabbit and goat anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. ECL Western Blotting Detection Reagents were purchased from GE Healthcare (Piscataway, NJ, USA). All other chemicals and reagents were purchased from Sigma Chemical Co.

4.3. Experimental Design

The Guidelines for Animal Experimentation approved by Daegu Haany University (permission No. 2013-036) were followed in the present study. Six-week-old male Sprague-Dawley rats were obtained from Daehan-Bio (Chungcheong, Korea) and were housed with room temperature and humidity maintained automatically at about 25°C and 55%, respectively. All rats were allowed free access to laboratory pellet chow (CLEA Japan, Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipids, and 60.5% carbohydrates) and water. After several days of adaptation, the rats were injected intraperitoneally with STZ (50 mg/kg body weight) in 10 mM citrate buffer (pH 4.5). The blood glucose level was determined and body weight was measured 10 days after the injection. To avoid any intergroup differences in these indices, the rats were divided into three experimental groups: group 1, diabetic rats receiving water (n=5); groups 2 and 3, diabetic rats receiving 10 or 20 mg/kg body weight/day of oligonol (n=5, each group) through once-daily oral gavage. The administration dose and duration were determined based on other reports [12-14]. Rats that underwent a sham injection of citrate buffer without STZ were used as a nondiabetic control group (n=5). After 10 days of treatment, blood samples were obtained from the abdominal aorta

under pentobarbital anesthesia (50 mg/kg body weight, intraperitoneally), and serum was prepared by centrifugation. Subsequently, each rat was perfused with ice-cold physiological saline and the pancreas was harvested, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

4.4. Estimation of Serum Parameters

Serum glucose was determined using a commercial kit (Glucose CII-Test; Wako Pure Chemical Industries, Ltd.). Serum C-peptide (Shibayagi Co., Ltd., Gunma, Japan) and insulin (Morinaga Institute of Biological Science, Yokohama, Japan) levels were estimated based on enzyme-linked immunosorbent assays. Serum ROS levels were determined according to the method of Ali et al. [41], and thiobarbituric acid-reactive substance (TBARS) levels were determined according to the method of Naito and Yamanaka [42].

4.5. Measurement of Insulin Content in the Pancreas

The insulin content of pancreatic tissue was estimated using the method of Portha et al. [43]. In brief, the pancreas was homogenized for 1 min by ultrasonic disintegration at 4°C in acid-alcohol solution (75% ethanol, 1.5% v/v 12 mol/L HCl, 23.5% distilled water) and the homogenate was maintained at -20°C overnight. Next, the extract was centrifuged and the insulin concentration of the supernatant was estimated by immunosorbent assay.

4.6. Determination of ROS Generation and TBARS Level in the Pancreas

ROS generation was measured as described previously [41]. Pancreatic tissues were homogenized on ice with 1 mM EDTA-50 mM sodium phosphate buffer (pH 7.4) and 25 mM

DCFH-DA was added to the homogenates. After incubation for 30 min, changes in fluorescence values were determined at an excitation wavelength of 486 nm and emission wavelength of 530 nm. The pancreatic TBARS level was determined according to the method of Uchiyama and Mihara [44].

4.7. Preparation of Nuclear and Post-Nuclear Fractions

Nuclear protein extraction was carried out as described previously [45]. Briefly, pancreatic tissue was homogenized with ice-cold lysis buffer containing 5 mM Tris-HCl (pH 7.5), 2 mM MgCl₂, 15 mM CaCl₂, and 1.5 M sucrose, and 0.1 M dithiothreitol (DTT) and protease inhibitor mixture solution were subsequently added. After centrifugation ($10,500 \times g$ for 20 min at 4°C), the resulting pellet was suspended in extraction buffer containing 20 mM 2-[4-(2-hydroxyethyl)-1-piperazyl] ethanesulfonic acid (pH 7.9), 1.5 mM MgCl₂, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, and 0.1 M DTT and protease inhibitor mixture solution were subsequently added. The mixture was placed on ice for 30 min and the nuclear fraction was prepared by centrifugation at $20,500 \times g$ for 5 min at 4°C. The post-nuclear fraction was extracted from the pancreas of each rat, as described below. In brief, pancreatic tissue was homogenized with ice-cold lysis buffer (pH 7.4) containing 137 mM NaCl, 20 mM Tris-HCl, 1% Tween 20, 10% glycerol, 1 mM PMSF, and protease inhibitor mixture solution. The homogenate was centrifuged at $2,000 \times g$ for 10 min at 4°C. The protein concentration in each fraction was determined using a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA).

4.8. Immunoblotting Analyses

For the estimation of NF- κ Bp65 and histone protein expression, 10 μ g of protein from each

nuclear fraction was electrophoresed through a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). Separated proteins were transferred to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and incubated overnight with primary antibodies to NF- κ Bp65 or histone 4°C. After washing, blots were incubated with anti-rabbit or anti-mouse IgG HRP-conjugated secondary antibody for 1.5 h at room temperature. Next, the protein expression of insulin, p-p38, p-ERK1/2, p-I κ B α , COX-2, iNOS, TNF- α , IL-6, and β -actin was determined in 10 μ g of protein from each post-nuclear fraction, electrophoresed through 8–15% SDS-PAGE. Each antigen–antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with a Sensi-Q2000 Chemidoc system (Lugen Sci Co., Ltd., Seoul, Korea). Band densities were measured using ATTO densitograph software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to histone and/or β -actin. The protein levels of groups are expressed relative to those of nondiabetic rats (represented as 1).

4.9. Histological Examination

Excised sections of pancreatic tissue were fixed immediately in 10% neutral-buffered formalin and embedded in paraffin. Paraffin sections were cut and stained with hematoxylin-eosin (HE) prior to optical microscopy examination. The sections were viewed and photographed using a Moticam Pro 205A microscope (Moticam Pro 205A, Moticam Instruments Inc., Richmond, Canada) with Motic Images Advanced 3.2 software (Motic China Group Co., Ltd.).

4.10. Statistical Analysis

The data are expressed as means \pm SEM. Significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using SPSS 11.5.1 software for

Windows 2002 (SPSS Inc., USA). Values of $p < 0.05$ were considered significant.

5. Conclusions

Diabetic rats show decreased insulin secretion through pancreatic damage associated with the expressions of the oxidative stress-induced MAPK activation-derived proinflammatory signaling pathway (I κ B/NF- κ B pathway) and proinflammatory genes (COX-2, iNOS, TNF- α , and IL-6). These unfavorable outcomes were reversed by oligonol administration, which improved overall pancreatic β -cell function-related indices, such as serum C-peptide and insulin secretion, pancreatic insulin expression level, and morphological characteristics in diabetic rats (Figure 8).

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

Abbreviations

ANOVA	analysis of variance
COX-2	cyclooxygenase-2

DCFH-DA	2',7'-dichlorofluorescein diacetate
DTT	dithiothreitol
ERK1/2	extracellular signal-regulated kinase 1/2
HE	hematoxylin-eosin
HRP	horseradish peroxidase
I κ B α	inhibitor of NF- κ B α
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
MAPK	mitogen-activated protein kinase
NF- κ B	nuclear factor-kappa B
NO	nitric oxide
PMSF	phenylmethanesulfonyl fluoride
ROS	reactive oxygen species
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel
STZ	streptozotocin
TBARS	thiobarbituric acid-reactive substance
TNF- α	tumor necrosis factor- α

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Legends to figures

Figure 1. Body weight (A), food intake (B), water intake (C), and pancreatic weight (D). N, non-diabetic rats; Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean \pm SEM. Significance: * p <0.05, ** p <0.001 vs. vehicle-treated diabetic rat values.

Figure 2. Glucose (A), C-peptide (B), and insulin (C) levels in the serum. N, non-diabetic rats; Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean \pm SEM. Significance: * p <0.05, ** p <0.01, p <0.001 vs. vehicle-treated diabetic rat values.

Figure 3. Insulin level in the pancreas. N, non-diabetic rats; Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean \pm SEM. Significance: * p <0.001 vs. vehicle-treated diabetic rat values.

Figure 4. p-p38 (A) and p-ERK1/2 (B) protein expressions in the pancreas. N, non-diabetic rats; Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean \pm SEM. Significance: * p <0.05, ** p <0.01, *** p <0.001 vs. vehicle-treated diabetic rat values.

Figure 5. p-I κ B α (A) and NF- κ Bp65 (B) protein expressions in the pancreas. N, non-diabetic rats; Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean \pm SEM.

Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle-treated diabetic rat values.

Figure 6. COX-2 (A), iNOS (B), TNF- α (C), and IL-6 (D) protein expressions in the pancreas. N, non-diabetic rats; Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean \pm SEM. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle-treated diabetic rat values.

Figure 7. HE staining of pancreatic tissues. Original magnification x200. N, non-diabetic rat; Veh, vehicle-treated diabetic rat; O10, oligonol 10 mg/kg body weight-treated diabetic rat; O20, oligonol 20 mg/kg body weight-treated diabetic rat. Scale bar is 100 μ m.

Figure 8. Possible mechanism of the effect of oligonol on the hyperglycemia-induced pancreatic damage.

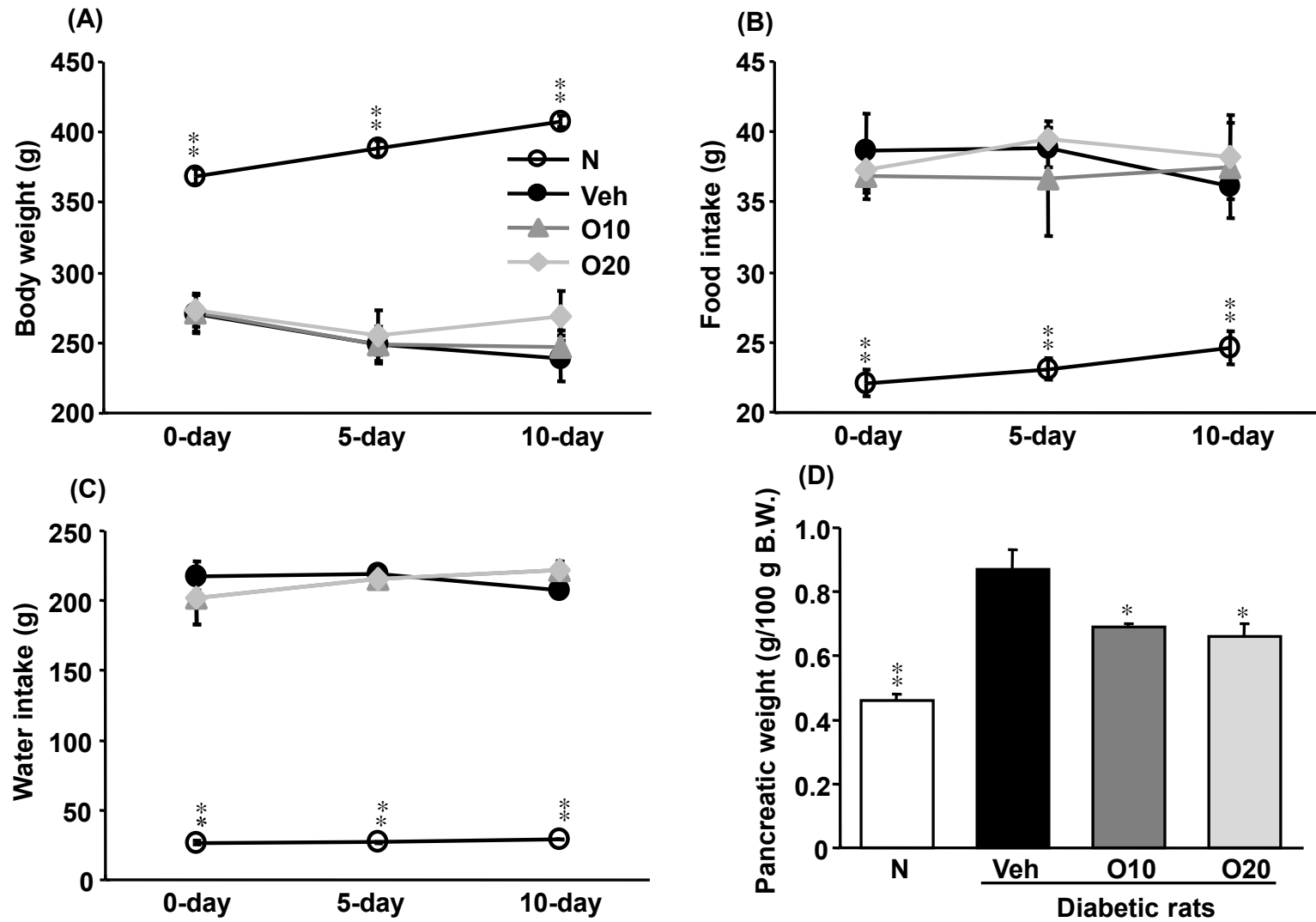


Figure 1.

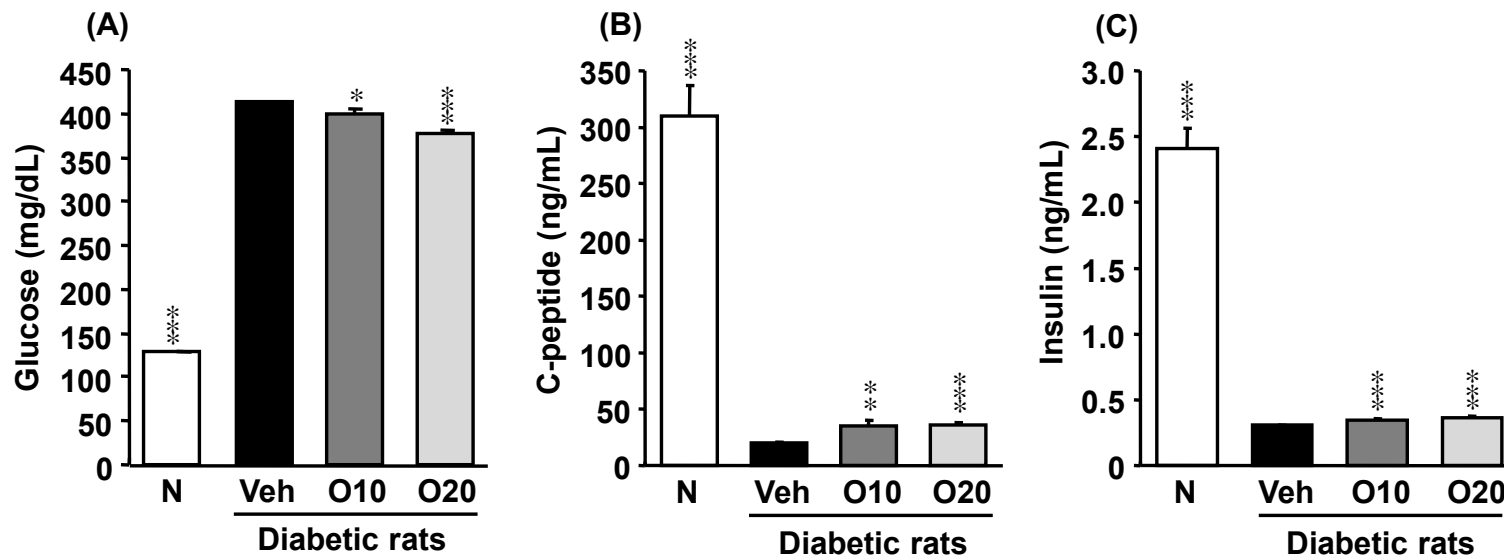


Figure 2.

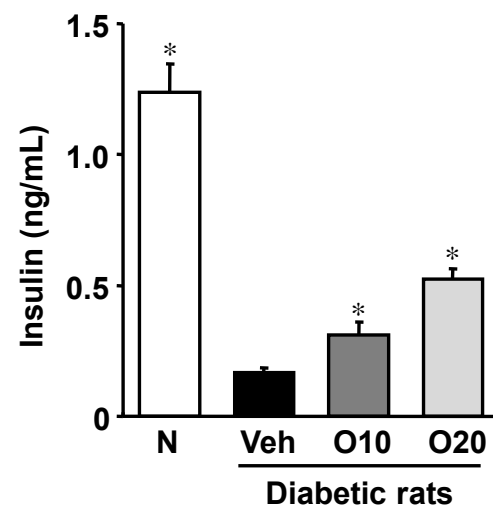


Figure 3.

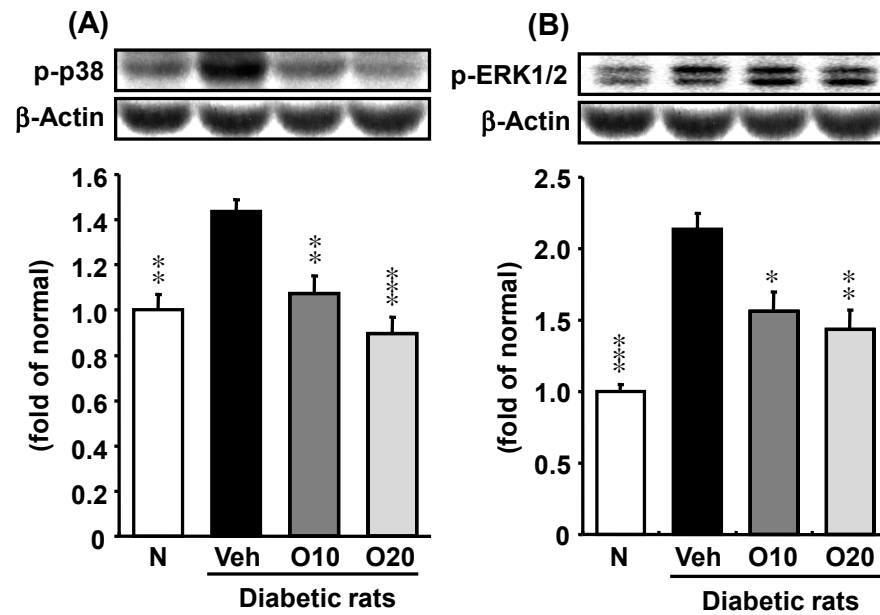


Figure 4.

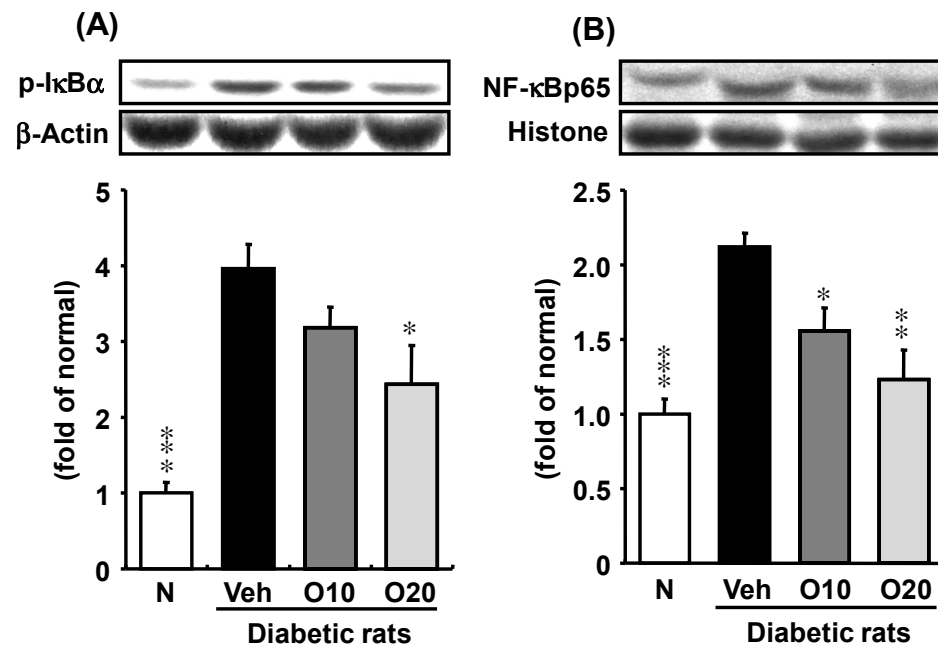


Figure 5.

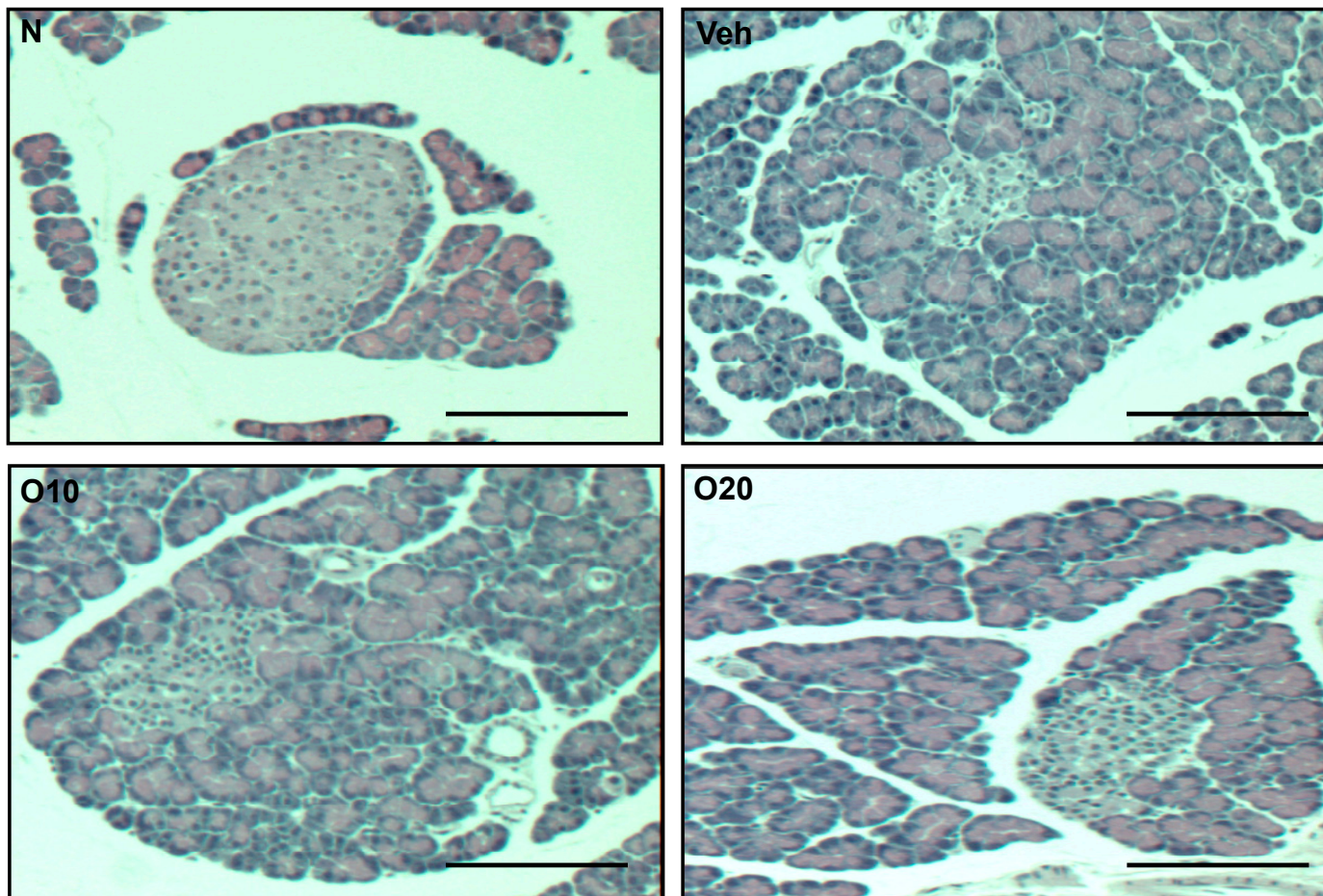


Figure 7.

N	<u>veh</u>	<u>O10</u>	<u>O20</u>
	Diabetic rats		

N	<u>veh</u>	<u>O10</u>	<u>O20</u>
	Diabetic rats		

Figure 6.

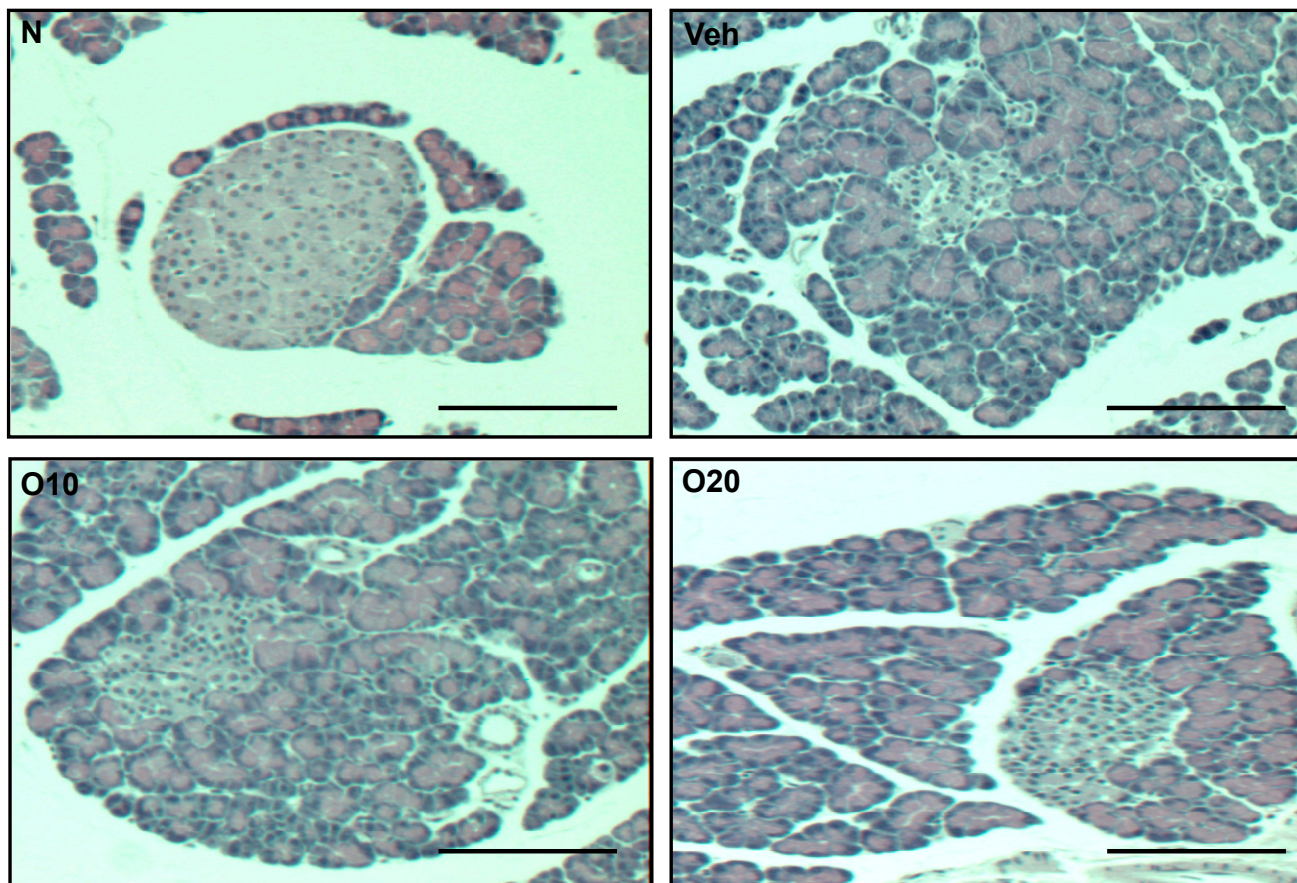


Figure 7.

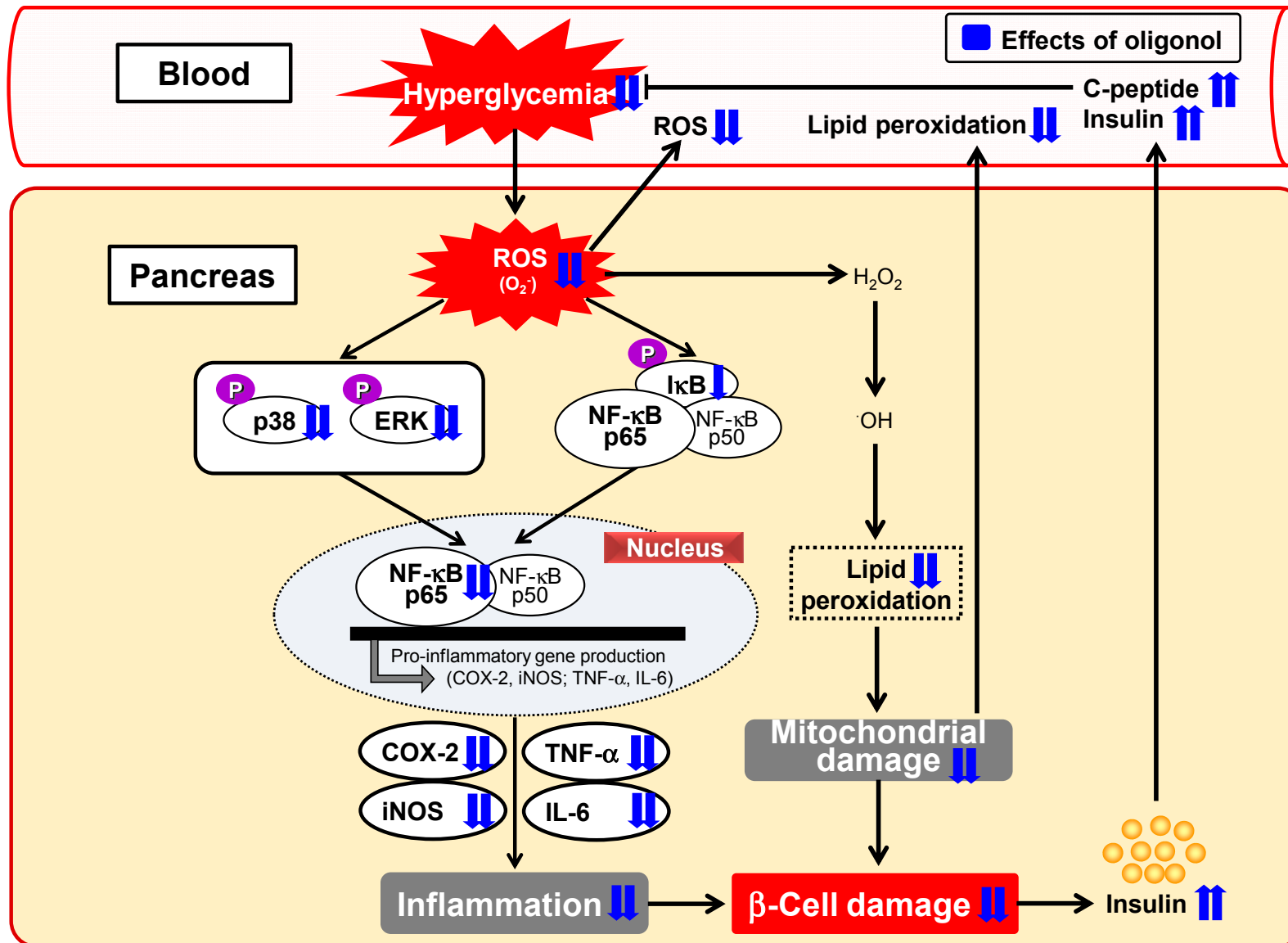


Figure 8.

Table 1. Biomarkers associated with oxidative stress in serum and pancreas.

Item	Non-diabetic rats	Diabetic rats		
		Veh	O10	O20
Serum				
ROS (fluorescence/min/mL)	34.2 ± 3.0 ^{***}	304.6 ± 29.8	210.4 ± 23.6 [*]	156.6 ± 23.6 ^{**}
TBARS (nmol/mL)	0.10 ± 0.01 ^{***}	0.24 ± 0.02	0.17 ± 0.02 [*]	0.15 ± 0.01 ^{**}
Pancreas				
ROS (fluorescence/min/mg protein)	10272 ± 95 ^{***}	13516 ± 291	12196 ± 598 [*]	12118 ± 218 ^{**}
TBARS (nmol/mg protein)	0.22 ± 0.01 ^{***}	0.41 ± 0.01	0.23 ± 0.01 ^{***}	0.23 ± 0.02 ^{***}

Veh, vehicle-treated diabetic rats; O10, oligonol 10 mg/kg body weight-treated diabetic rats; O20, oligonol 20 mg/kg body weight-treated diabetic rats. Data are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle-treated diabetic rat values.

Table 2. Phenol composition of oligonol used in the present study.

Phenols	Composition (%)
Monomers (Flavin-3-ols)	
Catechin + epicatechin	6.94
Epicatechin gallate	1.36
Epigallocatechin gallate	7.02
Dimers (procyanidins)	
Procyanidin A1	6.21
Procyanidin A2	6.59
Procyanidin B1	0.43
Procyanidin B2	1.98
Epicatechin-epigallocatechin gallate	1.45
Trimer	
Epicatechin-procyanidin A2	4.53
Other phenolic compounds	54.98
Total polyphenols	91.49
Miscellaneous (water, proteins, fats, etc.)	8.51