KSÜ Tarım ve Doğa Derg 24 (4): 689-700, 2021
KSU J. Agric Nat 24 (4):689-700, 2021
DOI:10.18016/ksutarimdoga.vi.811253

Protective Effects of Lyophilized Ethanolic Extract of Achillea arabica Kotschy. on the Islet ß Cells of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

In this study, an evaluation of the protective effect of lyophilized ethanolic *Achillea arabica* Kotschy. extract was investigated on the islets of Langerhans in streptozotocin (STZ)-induced diabetic rats. The rats were divided randomly into 5 groups: Group I: control; Group II: rats treated with STZ (50 mg kg^{-1} body weight (bw)); Group III: rats treated with *A. arabica* (Aa) extract (400 mg kg^{-1} bw); Group IV: rats with DM treated with Aa extract; and Group V: rats with DM treated with glibenclamide (2 mg kg^{-1} bw) which is a standard drug used in the treatment of DM. STZ treatment caused histopathological changes in the islet and significantly lowered the islet diameter/area, β-cell index values, and blood insulin and C-peptide levels. Administration of the extract of Aa improved the islet histology, diameter/area, and β-cell index values, and caused significant increases in insulin and C-peptide levels similar to the rats treated with glibenclamide when compared to the diabetic rats. Our findings suggested that the Aa extract possessed protective potential on the pancreatic islets due to its repairing or regeneration impact on β-cells.

Reserach Article

Article History
Received : 15.10.2020
Accepted : 03.12.2020

Keywords
Streptozotocin
Diabetes
Rat
Achillea arabica
Histopathology

Achillea arabica Kotschy. Liyolifilize Etanolik Ekstresinin Streptozotosin ile Diyabet Oluşturulan Ratların Adacık β Hücreleri Üzerindeki Koruyucu Etkileri

ÖZET

Bu çalışmada, *Achillea arabica* Kotschy. liyofilize etanolik ekstresinin, streptozotosin (STZ) ile diyabet oluşturulan ratlarda Langerhans adacıkları üzerindeki koruyucu etkisi araştırıldı. Ratlar rastgele olarak 5 gruba ayrıldı; Grup I: kontrol; Grup II: STZ (50 mg kg^{-1} vücut ağırlığı (va)) uygulanan ratlar; Grup III: *A. arabica* (Aa) ekstresi (400 mg kg^{-1} va) ile muamele edilen ratlar; Grup IV: Aa ekstresi ile muamele edilen diyabetik ratlar ve Grup V: diyabet tedavisinde standart bir ilaç olarak kullanılan glibenklamid (2 mg kg^{-1} va) uygulanan diyabetik ratlar. STZ uygulamasının, adacıklarda histopatolojik değişimlere neden olduğu, adacık çapını / alanını, β-hücre indeksini ve kan insulin ve C-peptid seviyelerini anlamlı olarak düşürdüğü belirlendi. Aa ekstre uygulamasının, glibenklamid ile tedavi edilen ratlara benzer olarak, adacık histolojisinde, çap / alanında, β-hücre indeks değerlerinde, kan insulin ve C-peptid seviyelerinde, diyabetik ratlar ile kıyaslandığında, iyileşmeye neden olduğu gözlendi. Elde edilen bulgular Aa ekstresinin pankreas adacıklarında, β-hücre onarımını ve yenilenmesini sağlayarak, koruyucu potansiyele sahip olduğunu göstermektedir.

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 15.10.2020
Kabul Tarihi : 03.12.2020

Anahtar Kelimeler
Streptozotosin
Diyabet
Rat
Achillea arabica
Histopatoloji

To Cite : Hanalp HC, Kaptaner B, Doğan A 2021. Protective Effects of Lyophilized Ethanolic Extract of Achillea arabica Kotschy. on the Islet ß Cells of Streptozotocin-Induced Diabetic Rats. KSU J. Agric Nat 24 (4): 689-700. DOI: 10.18016/ksutarimdoga.vi.811253.

INTRODUCTION

Diabetes mellitus (DM), which is a grave metabolic disorder, is defined by hyperglycemia associated with carbohydrate, protein, and lipid metabolism

abnormalities, and is one of the most serious diseases affecting public health globally. DM was reported as the cause of 1.5 million deaths in 2012 by the World Health Organization. It is estimated that the number of people suffering from DM will reach 642 million by 2040. Although the main cause of DM is unknown thus far, researchers believe that the disease is related to genetic and environmental factors, modern life style, unhealthy diet, and a sedentary life. The symptoms of the disease comprise polyuria, polydipsia, polyphagia, weight loss, blurred vision, hyperglycemia and hypertension. The acute complications of DM accompany ketoacidosis or non-ketotic hyperosmolar syndrome, hyperosmolar coma, and diabetic coma. As a result of long-standing DM, high plasma glucose causes blood vessel damage leading to microvascular and macrovascular diseases. Microvascular complications include retinopathy, nephropathy, and neuropathy, while macrovascular diseases lead to cardiovascular diseases and diabetic foot. The classification of DM falls into 3 categories: Type 1 DM (T1DM) is dependent on insulin, Type 2 DM (T2DM) is not dependent on insulin, and Type 3 DM (T3DM) is gestational. T1DM is an autoimmune disease, in which insulin deficiency occurs because of islet β-cell destruction. T2DM is defined by resistance to insulin and impaired insulin secretion. T3DM, which is diagnosed during pregnancy, in the 2nd or 3rd trimester, is impermanent and might be an indication of T2DM (for reviews, see Jarald et al., 2008 and Okur et al., 2017). Chronic hyperglycemia generates reactive free radicals and finally, causes oxidative stress, which plays a pivotal role in the appearance of DM and its complications, leading to pancreatic β-cell damage (Giacco and Brownlee, 2010; Dos Santos et al., 2019). Actually, commercially available drugs for the treatment of DM exist; however, they have limitations because of their high cost and side effects. Therefore, there is a growing interest in herbal medicines for the treatment of DM that possess hypoglycemic, antioxidant, and antidiabetic properties and have few or no side effects (Jarald et al., 2008).

The genus *Achillea* L. (Asteraceae) comprises ~85 species, 42 of which are found in Turkish flora, and 23 are endemic (Huber-Morath, 1975; Duman, 2000). Many species belonging to the genus *Achillea* L. possess medicinal properties and are used for therapeutic purposes in some countries (Bashi et al., 2012). In Turkey, some *Achillea* species have been traditionally used to treat different diseases, such as abdominal pain (Honda et al., 1996), diarrhea (Yesilaada et al., 1993), stomachache, and wound healing (Fujita et al., 1995; Sezik et al., 2001). The antioxidant, antimicrobial, antitumoral, antidiabetic, hypoglycemic, antiulcer, antileishmanial, and antinematodal activities, and wound healing impact of different *Achillea* species have been reported by different researchers (Conforti et al., 2005; Konyalioglu and Karamenderes, 2005; Bariş et al., 2006; Bashi et al., 2012; Zolghadri et al., 2014; Motavalizadehkakhky et al., 2013; Al-Sokari, 2015; Baharara et al., 2015; Varasteh-Kojourian et al., 2015; Al-Marby et al., 2016; Al-Said et al., 2016, Abd El-Fattah et al., 2018). *Achillea arabica* Kotschy. (Syn.: *Achillea biebersteinii* Afanasiev) has also been observed to possess antioxidant, DNA-protective (Varasteh-Kojourian et al., 2015), hepatoprotective (Al-Said et al., 2016), wound healing (Akkol et al., 2011), antiulcer, and hypoglycemic (Abd-Alla et al., 2016) properties; however, its pancreatic islet-protective and antidiabetic capabilities, and underlying mechanisms, are unknown.

Accordingly, herein, it was aimed to investigate the protective effects of lyophilized ethanolic flower extract of *A. arabica* (Aa) in STZ-induced diabetic rats using histopathology, β-cell immunohistochemistry, and blood insulin/C-peptide levels.

MATERIALS and METHODS

Plant Material

The Aa used in this study was collected from Edremit, Van, Turkey (43°15′54″E, 38°25′09″N). The plant samples were taxonomically identified and approved by a plant taxonomist, Süleyman Mesut Pinar (PhD), at Van Yuzuncu Yil University, and the voucher specimens were placed into the Van Yuzuncu Yil University, Faculty of Science, Department of Biology Herbarium (VANF), under number 164098. The collected samples were washed carefully with distilled water and laid out to dry at room temperature in the dark. Next, the flower parts of the dried samples were finely ground using a blender and the powdered samples were kept at –20 °C while awaiting the extraction procedures.

Plant Extraction

Extraction of the lyophilized ethanolic was performed according to the methods of Dogan et al., (2018), with some modifications. In brief, 50 g of the ground and powdered dry material from the inflorescence was mixed with an ethanol:water (80:20) solvent (1 L), for 2 h at room temperature, by continuous shaking, and then filtered. Next, the filtrate was centrifuged at 10,000 rpm for 20 min at 4 °C, and the supernatant was then collected. Filtering of the supernatant took place via passing it through a 0.45-µm filter, and the solvent was removed by evaporation at 37 °C under decreased pressure. The viscous extract was allowed to falcon tube and freeze-dried under a vacuum at –51 ºC to obtain a fine lyophilized powder.

Animals

A total of 40 *Wistar albino* female rats, which were 2–

3 weeks old and weighed 100–350 g, were acquired from the Experimental Animal Research Center of Van Yuzuncu Yil University. The rats were housed in cages made of plastic at a steady room temperature of 25 ± 2 °C and a 12-h light/dark photoperiod. They were supplied with drinking water and a diet that was wheat and soybean meal-based *ad libitum*.

Ethical Approval

The animals used, and all of the experimental procedures during the study, were approved of by the Animal Experiments Ethics Committee of Van Yuzuncu Yil University for ethical concerns (Decision no: VAN YUHADYEK-2019/06). During the experiments, the ethical regulations that were followed were in line with the institutional and national guidelines for animal welfare protection.

Acute Toxicity Test

The acute toxicity test study was employed according to the method of Ibeha and Ezeaja, (2011) as described by Doğan and Çelik, (2016). For evaluation of the acute toxicity of the lyophilized ethanolic Aa extract on the rats, increasing dosages of 25, 50, 100, 250, 500, 1000, and 2000 mg kg^{-1} of body weight (bw) were given via oral gavage for 0, 0.4, 4, 8, 12, 24, 48 and 72 h, respectively. Signs or symptoms of toxicity and mortality were not determined for the first 72 h. Thereafter, the favorable dosage of the extract used in the experimental study was ascertained to be 400 mg kg^{-1}.

Induction of the Diabetes and Experimental Design

Randomly, the rats were divided into 5 groups, comprising 7 rats in each.

Group I: Control (C) group, where the rats received a 4.5-pH citrate buffer at a dose of 1 mL kg^{-1} bw, administered via intraperitoneal (i.p.) injection.

Group II: DM group, where the rats were fasted for 12 h and then administered a single dose of freshly prepared streptozotocin (STZ) in a citrate buffer comprising 50 mg kg^{-1} bw via i.p. injection. On day 3, at 72 h following administration of the STZ, samples of blood were taken from the tail vein of the rats and glucometer test strips (Accu-Chek Go, Roche Diagnostics, Istanbul, Turkey) were used to measure glucose levels. Rats that had blood glucose levels higher than 200 mg dL^{-1} were accepted as diabetic, and implicated in the study.

Group III: Aa group, where the rats were administered a single daily dose of lyophilized Aa extract (400 mg kg^{-1} bw) via oral gavage for 21 days.

Group IV: DM + Aa group, where lyophilized Aa extract (400 mg kg^{-1} bw) was administered daily to rats with DM via oral gavage for 21 days.

Group V: DM + glibenclamide (Gli) group, where Gli (2 mg kg^{-1} bw) as a reference drug was administered daily to rats with DM via oral gavage for 21 days. The dosage of the drug was selected according to Sokolovska et al., (2012) and Okwudili et al., (2017).

Tissue Sampling and Histological Procedures

After completion of the experimental study, the animals were anesthetized intraperitoneally with ketamine (5 mg 100 g^{-1} bw). Blood samples were taken via cardiac puncture with sterile injectors. Next, the samples were immediately transferred into EDTA-coated tubes. The tubes were then centrifuged for 15 min at 4000 *g* and 4 °C and the plasma was obtained and then stored at -80 °C until the analyses.

The pancreas of each animal was excised carefully, then fixed with 10% neutral buffered formalin for 24 h at 4 °C. The tissues were then washed with phosphate-buffered saline at a pH of 7.4, and passed through a graduated ethanol series for dehydration. After clearing with xylene, they were embedded in paraffin wax. Longitudinal tissue sections (5-µm thick) were taken using a manual rotary microtome (H315, Microm GmbH, Waldrof, Germany) and put onto slides coated with adhesive (Marienfeld GmbH, Lauda-Königshofen, Germany). Sections were deparaffinized with xylene, then rehydrated through decreasing ethanol concentrations. After staining with Hematoxylin and Eosin (H&E), Canada balsam (Merck, Germany), was used to mount all of the preparations and a microscope was used to examine and photograph them (Leica DMI 6000 B, Leica Microsystems CMS GmbH, Wetzlar, Germany).

Insulin Immunohistochemistry

The 5-µm-thick tissue sections were first deparaffinized and then rehydrated, and hydrogen peroxide was then used to incubate them for 10 min, so as to prevent internal peroxidase activity. As a next step, the sections were washed in tris buffered saline (TBS; pH: 7.6, 20 mM Tris, 140 mM NaCl) and, to avoid the occurrence of nonspecific binding, a protein a protein block, supplied in the immunohistochemistry kit used in the onward steps was applied for 10 min. The sections were then incubated with insulin (insulin monoclonal antibody (INS05 (2D11-H5)), catalog no: MA5-12037, Invitrogen, Thermo Fisher Scientific, Illinois, USA) primary antibody at a dilution rate of 1:25, at 4 °C for 24 h. The commercial EXPOSE mouse and rabbit specific IHC/DAB detection immunohistochemistry kit (catalogue number: ab80436, Abcam, Cambridge, UK) was used, following the manufacturer's instructions, for the next steps. First, the sections were rinsed with TBS and they were then incubated, using the complement supplied in the kit, for 10 min, followed by treatment with a conjugate of horseradish peroxidase. Visualization of the

peroxidase activity was performed using a substrate-chromogen solution containing 3,3'-diaminobenzidine, which formed a brown stain, that was then used for section incubation for 1 to 3 min, after which, they were washed using water that was distilled twice. Counterstaining was performed using Mayer's hematoxylin. Next, the sections were dehydrated through 95 and 100% ethanol, cleared in xylene and then mounted with Canada balsam and then covered with cover slips. For testing the antibody specificity, negative control slides were utilized, in which TBS was used in place of the primary antibodies (Kaptaner, 2019). A Leica DMI 6000 B (Leica Microsystems CMS GmbH, Wetzlar, Germany) microscope was used to both examine and photograph the preparations.

Morphometric Measurements and β-Cell counting

A total of 8 pancreas optical areas were randomly chosen, that has no points of intersection, and were photographed with a Leica Digital DFC490 camera (Leica Microsystems) attached to a Leica DMI 6000B model microscope at a magnification of 400× for each of the insulin immuno-stained sections. ImageJ software (National Institutes of Health, USA, http://rsbweb.nih.gov/ij/) was used to process the images that were captured.

The diameter or area measurements of the pancreatic islets were performed using photomicrographs captured at magnifications of 200× and 400×, as described in the ImageJ User Guide (version revised edition IJ 1.46r) via the analyze tool. At least 8 randomly selected Langerhans islets pertaining to each animal were used for both of the measurements. The measurement of diameter of pancreatic islets was carried out as described by Dra et al., (2019) with some modifications. Briefly, the mean diameters for each islet in a section was determined, and then the mean islet diameter was calculated from the means of the islet diameters for each animal. Finally, the value pertaining to each animal within the same group was used for statistical analyses. The same procedure was used for the area measurements.

Cell counting in the islets for the β-cell index calculations comprised at least 8 Langerhans islets for each section from each animal. Cell counting in the islets was done using the cell counter plugin of ImageJ Software (https://imagej.nih.gov/ij/plugins/cell-counter.html). The β-cell index for each islet was calculated as follow:

$$\beta\text{-cell index (\%)} = \frac{\text{Insulin positive cells in islet}}{\text{Total cells (insulin negative cells + insulin positive cells) in islet}} \times 100$$

Finally, the mean of the β-cell index value was calculated from the means obtained from the islets for each animal.

Plasma Insulin and C-Peptide Levels

Commercial kits (rat insulin enzyme linked immunosorbent assay (ELISA) kit, catalogue no: YHB0584Ra rat and C-peptide ELISA kit, catalogue no: YHB0281Ra, respectively; Shangai Yehua Biological Technology, Co., Ltd., Shangai, China) were used for the measurement of the plasma insulin and C-peptide. The assays were performed according to the manufacturer's instructions in the kit booklets.

Statistical Analyses

SPSS v.16.0 (IBM Corp., Armonk, NY, USA) was used for the statistical evaluation of the data from the measurements. Duncan's multiple comparison post hoc test and one-way analysis of variance was used to analyze differences among the data. The results were expressed as the mean ± standard error of the mean (SE). $P<0.05$ was considered as statistically significant.

RESULTS and DISCUSSION

Histological examinations of H&E stained pancreas sections from the control group displayed the normal appearance of the Langerhans islets, where they had well-defined borders, a rich capillary supply, and normal β- and α-cell contents (Fig 1A). The number of islets of the STZ-induced diabetic rats was observed to be dramatically reduced, and likewise, the shape and size of the islets was distorted or shrunken. The islet β-cells revealed degenerative and necrotic changes, where they mostly exhibited low staining with eosin or unstained vacuolations in their cytoplasms and their nuclei were stained dark and determined to be condensed, exhibiting pyknotic features (Fig 1B). Few histopathological changes were observed in either the DM + Aa or DM + Gli groups. Improved histological architecture and integrity of the islets were found in the DM + Gli group compared to the DM group (Fig 1C). Similarly, we found improvement in the Langerhans islets of the DM + Aa group compared to the DM group that the size and number of islets were increased and β-cell recovery was observed (Fig 1D).

The morphometric measurements conducted in the Langerhans islets showed that STZ induction caused a significant decrease (109.08 ± 5.54 μm) in the diameter of the islets when compared to the control (188.34 ± 11.59 μm) and Aa (178.60 ± 8.27 μm) groups ($P < 0.05$). A significant increase (130.15 ± 5.82 μm; $P < 0.05$) in the islet diameter was observed in the DM + Aa group when compared to the DM group; however, this increase was less effective than those measured in the islets of the DM + Gli group (138.61 ± 7.96 μm; $P < 0.05$) (Fig 2).

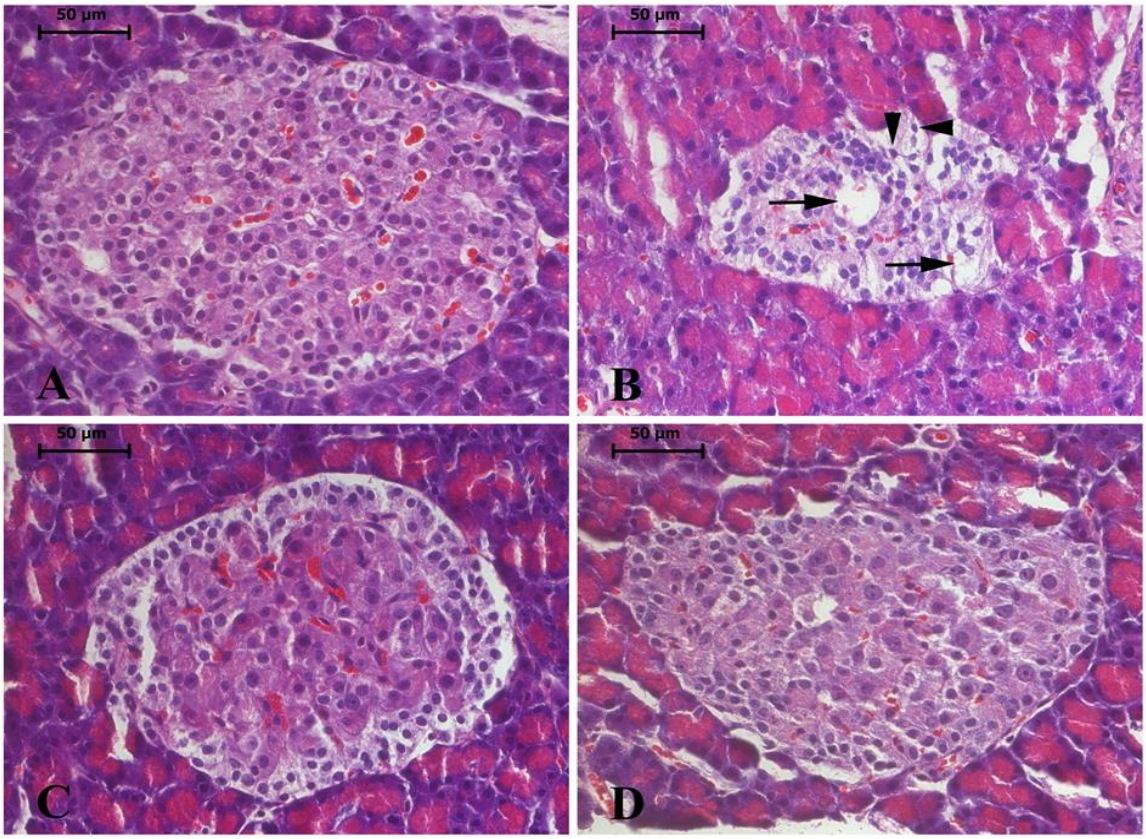

Figure 1. Pancreas sections stained with H&E from the different experimental groups. A) Islet of Langerhans from control group showing normal architecture and integrity. B) Distorted or shrunken islet of Langerhans of a diabetic animal from the DM group showing β-cell loss, cytoplasmic vacuolation (arrows) and darkly stained and condensed nuclei (arrowheads). C) Islet of Langerhans from a Gli-treated animal showing amelioration of diabetic features. D) Islet of Langerhans from an Aa treated diabetic animal showing a marked improvement.

Şekil 1. Farklı deneysel gruplara ait H&E ile boyanmış pankreas kesitleri. A) Kontrol grubuna ait normal yapı ve bütünlük gösteren Langerhans adacığı. B) DM grubuna ait diyabetik bir hayvanın, β-hücre kaybı, sitoplazmik vakuolizasyon (oklar) ve koyu veya yoğun boyanmış nukleuslara sahip olan (ok başları) bozulmuş ve büzülmüş Langerhans adacığı. C) Diyabetik özeliklerin iyileşme gösterdiği glibenklamid ile muamele edilen bir hayvana ait Langerhans adacığı. D) Aa ile muamele edilmiş bir diyabetik hayvanın, belirgin bir iyileşme gösteren, Langerhans adacığı.

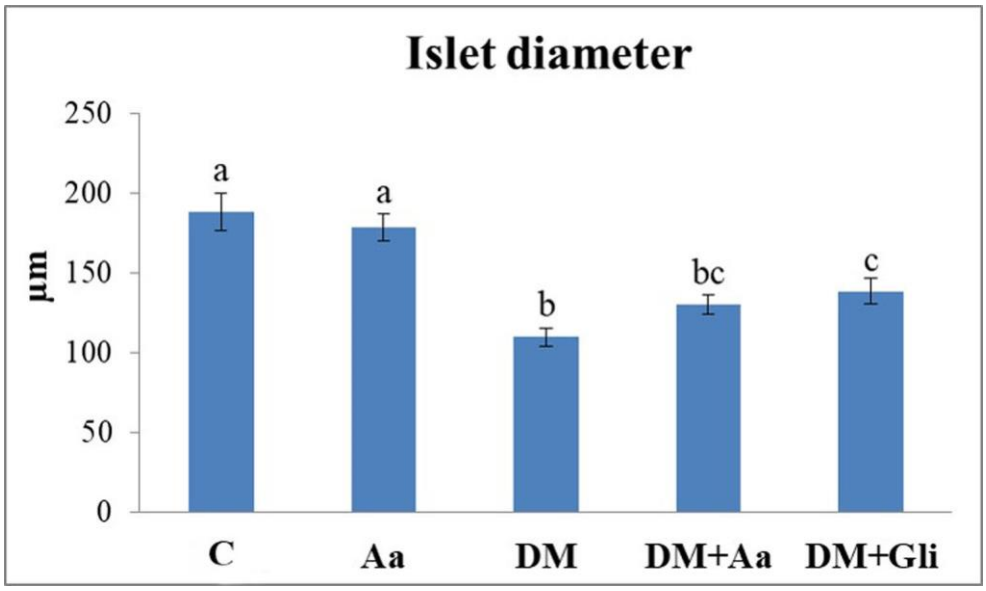

Figure 2. The mean islet diameters for the experimental groups. Data are means ± SE, (n = 6). Different letters indicate significant differences between the columns.

Şekil 2. Deneysel gruplara ait ortalama adacık çapları. Veriler ortalama ± standart hata olarak sunulmuştur (n = 6). Farklı harfler, sütünlar arasındaki anlamlı farklılıkları göstermektedir.

The same trend was also found in the measurement of the mean islet area. As shown in Fig 3, the mean islet area significantly decreased (10.51 ± 1.56 mm²) in the DM group when compared to the control (31.61 ± 3.01 mm²) and Aa (29.35 ± 1.82 mm²) groups (P < 0.05). The islet area was significantly increased in both the DM + Aa and DM + Gli groups (16.08 ± 1.32 mm² and 16.76 ± 1.99 mm², respectively; P < 0.05) when compared to those observed in the DM group. The increase in the islet area in the DM + Aa group was significantly low when compared to that of the DM + Gli group (P < 0.05). On the other hand, both the mean islet diameter and area values in the DM + Aa and DM + Gli groups were still significantly lower than the levels found in the NC and Aa groups (P < 0.05).

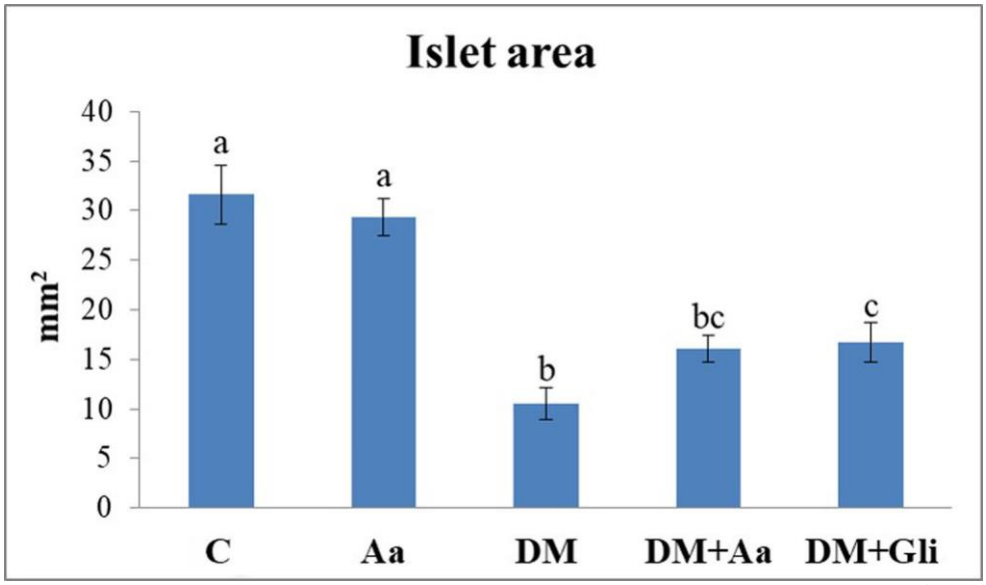

Figure 3. The mean islet areas for the experimental groups. Data are means ± SE, (n = 6). Different letters indicate significant differences between the columns.

Şekil 3. Deneysel gruplara ait ortalama adacık alanları. Veriler ortalama ± standart hata olarak sunulmuştur (n = 6). Farklı harfler, sütünlar arasındaki anlamlı farklılıkları göstermektedir.

Immuno-stained sections from the control (Fig 4A) and Aa groups (not shown) displayed a strong cytoplasmic immune-reactivity of β-cells, which are located mainly in the core of the Langerhans islets. In rats with DM, no or a few sporadic immune-positive β-cells were observed in the islets, and some of them exhibited a vacuolated cytoplasm (Fig 4B). A marked reconstitution in the immune-positive β-cells was detected in the DM + Gli and DM + Aa groups (Figs 4C and 4D, respectively). In accordance with those observations, the β-cell index was dramatically reduced in the DM group (5.95 ± 1.01%) when compared to the control (68.13 ± 3.06%) and Aa (68.33 ± 4.14%) groups (P < 0.05). Significant increases in the DM + Gli and DM + Aa groups (43.70 ± 6.53% and 28.37 ± 11.27%, respectively; P < 0.05) were revealed in the β-cell index when compared to the DM group. However, the β-cell index values in both the DM + Aa and DM + Gli groups were still significantly lower than those determined in the control and Aa groups (P < 0.05) (Fig 5).

The plasma insulin level exhibited a significant decrease in the DM group (0.72 ± 0.19 mIU/L) when compared to the control (1.72 ± 0.30 mIU/L) and Aa (1.94 ± 0.16 mIU/L) groups (P < 0.05). In both the DM + Aa and DM + Gli groups, insulin levels increased significantly (1.38 ± 0.20 mIU/L and 2.04 ± 0.12 mIU/L, respectively) to near normal levels compared to the DM group (Fig 6). The plasma C-peptide level was also considerably decreased in the DM group (135.03 ± 48.91 pg/mL, P < 0.05) when compared to the control (794.00 ± 114.74 pg/mL) and Aa (719.68 ± 32.28 pg/mL) groups. In both the DM + Aa and DM + Gli groups, significant increases in the plasma C-peptide levels (365.43 ± 107.98 mIU/L and 927.78 49.56 ± 0.12 mIU/L, respectively) were observed when compared to the DM group. The DM + Gli group exhibited plasma C-peptide levels near normal concentration, whereas for DM + Aa group, the Aa extract was less effective than Gli in returning the plasma C-peptide to normal levels (Fig 7).

DM is a metabolic disorder that has been reported worldwide, and is defined by hyperglycemia sourced from a lack of insulin production or deficiency of insulin action, or both, and the disease is still a significant cause of death. Chronic elevation of blood glucose levels leads to complications, including cardiovascular diseases, nephropathy, retinopathy, and neuropathy. Several plant extracts, such as phytochemicals or plant-derived natural compounds, are attractive agents that have been investigated

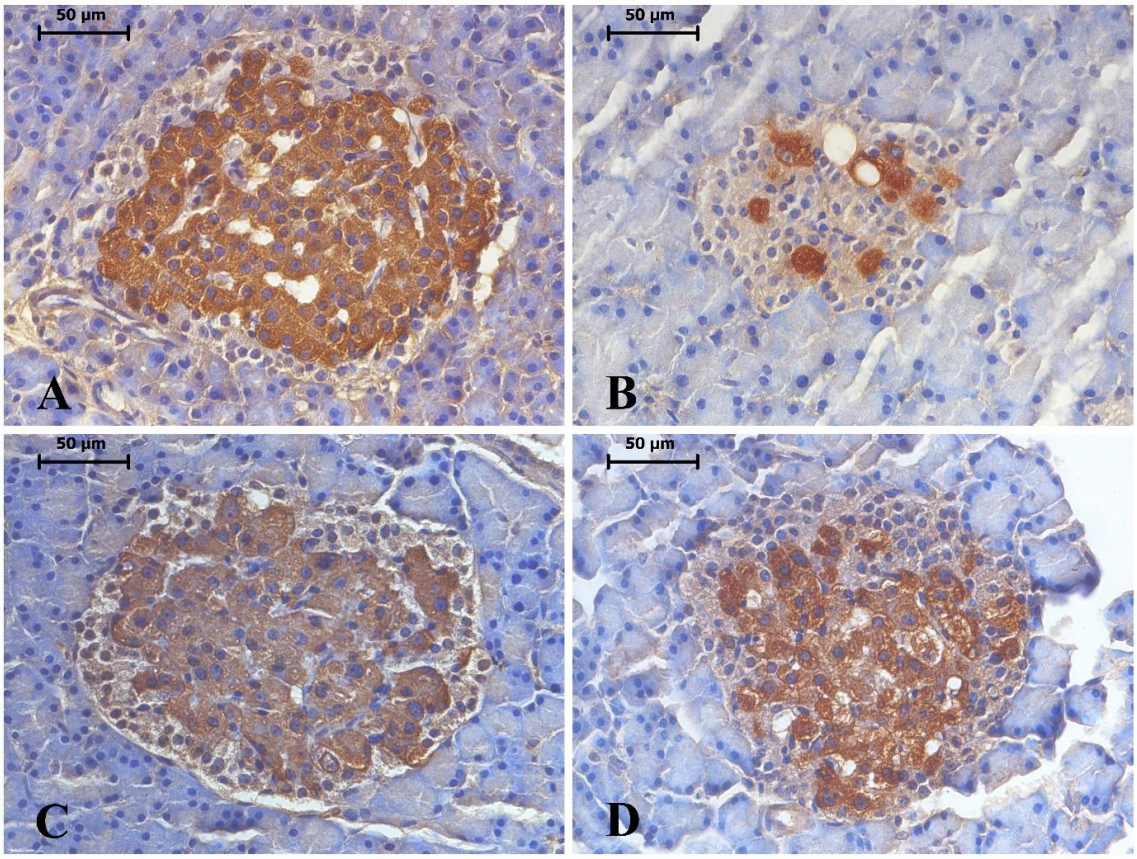

Figure 4. Pancreas sections immunostained with anti-insulin antibody. A) β-cells exhibit strong cytoplasmic immunostaining (brown) and occupying mainly the core of the islet from an animal from the control group. B) Decreased number of β-cells in a shrunken islet from the DM group; some cells exhibit cytoplasmic vacuolation. C) Islet of Langerhans from a Gli-treated animal showing increased number of β-cells compared to DM group. D) Islet of Langerhans from Aa treated diabetic animal showing reconstruction of β-cells comparing to the DM group.

Şekil 4. Anti-insülin antikoru ile immun boyanan pankreas kesitleri. A) Kontrol grubuna ait bir hayvanın, güçlü sitoplazmik immun boyanma gösteren (kahverengi) ve çoğunlukla adacık merkezini kaplayan β-hücreleri. B) DM grubuna ait büzüşmüş bir adacıkta az sayıdaki β-hücreleri; bazı hücreler sitoplazmik vakuolizasyon göstermektedir. C) DM grubuna kıyasla β-hücre miktarının artış gösterdiği, glibenklamid ile muamele edilen bir hayvana ait Langerhans adacığı. D) Aa ile muamele edilmiş bir diyabetik hayvanın β-hücrelerinin, DM grubuna kıyasla yeniden yapılanma gösterdiği, Langerhans adacığı.

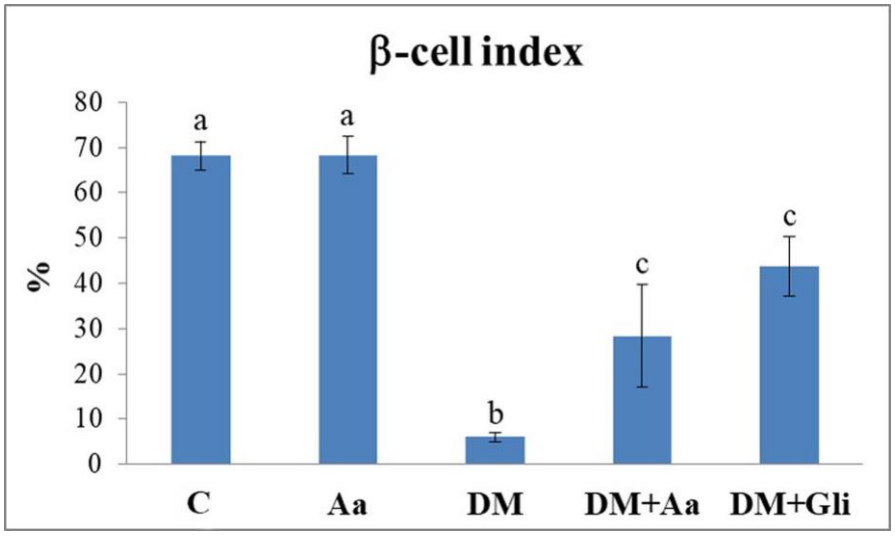

Figure 5. β-cell index values of the islets of Langerhans of different experimental groups. Data are means ± SE, (n = 6). Different letters indicate significant differences between the columns.

Şekil 5. Farklı deneysel gruplara ait Langerhans adacıklarının β-hücre indeks değerleri. Veriler ortalama ± standart hata olarak sunulmuştur (n = 6). Farklı harfler, sütünlar arasındaki anlamlı farklılıkları göstermektedir.

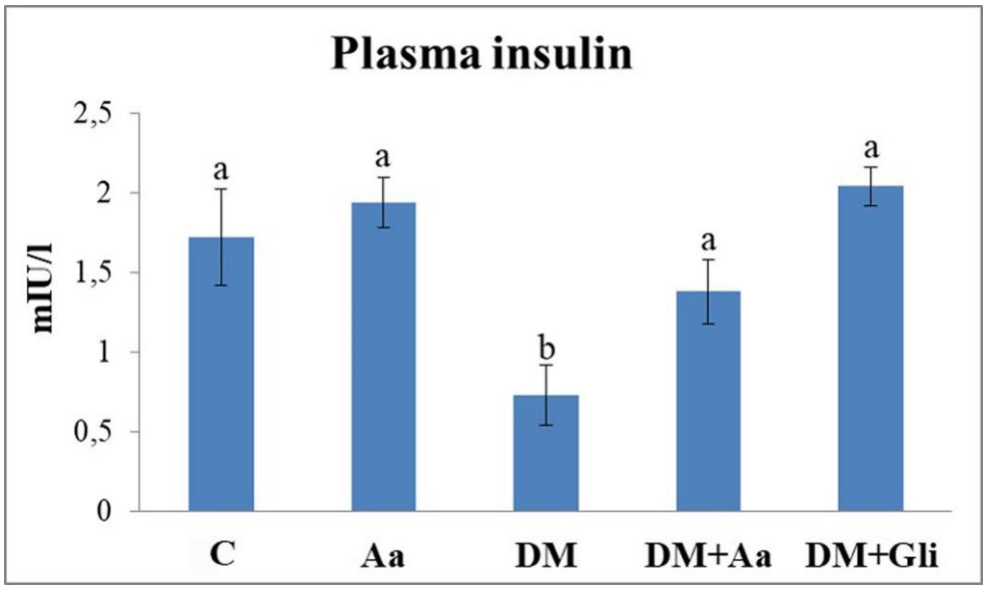

Figure 6. Plasma insulin levels for experimental groups. Data are means ± SE, (n = 6). Different letters indicate significant differences between the columns.

Şekil 6. Deneysel gruplara ait plazma insülin seviyeleri. Veriler ortalama ± standart hata olarak sunulmuştur (n = 6). Farklı harfler, sütünlar arasındaki anlamlı farklılıkları göstermektedir.

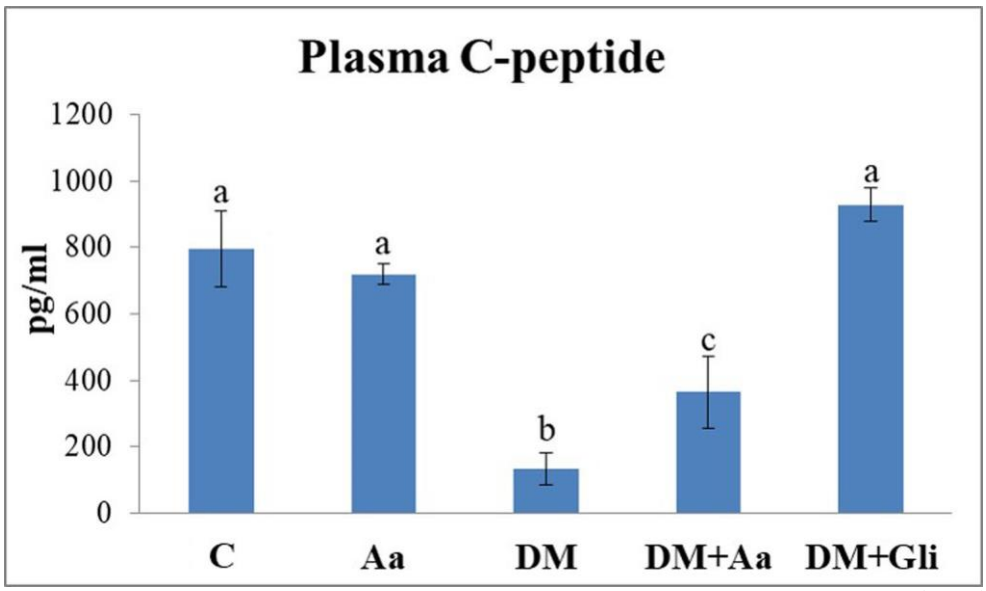

Figure 7. Plasma C-peptide levels for experimental groups. Data are means ± SE, (n = 6). Different letters indicate significant differences between the columns.

Şekil 7. Deneysel gruplara ait plazma C-peptid seviyeleri. Veriler ortalama ± standart hata olarak sunulmuştur (n = 6). Farklı harfler, sütünlar arasındaki anlamlı farklılıkları göstermektedir.

because of their ameliorative benefits on DM complications, especially over the last 2 decades (Bnouham et al., 2006; Hosseini et al., 2015; Oh, 2015). Members of the genus *Achillea* L. have been traditionally used in many countries for different purposes in the treatment of many diseases, such as fevers, hypertension, hemorrhage, hemorrhoid, wounds, abdominal pain, rheumatic pain, etc. (Hammad et al. 2013). Scientific research has shown that aerial parts of Aa, especially the flowers, have exhibited antioxidant and antitumoral capabilities (Baharara et al., 2015; Al-Said et al., 2016; Varasteh-Kojourian et al., 2017); however, its antidiabetic impact has been less studied and their actions of mechanisms on diabetic complications remain unknown. Therefore, the specific aim herein was an investigation of the protective effects of Aa flower ethanolic extract on pancreatic β-cells and its antidiabetic role in STZ-induced diabetic *Wistar albino* rats.

The histological and morphometric analyses performed in the H&E stained pancreas sections showed that the islets were shrunken and distorted by STZ. Moreover, degenerative and necrotic changes, such as cytoplasmic vacuolation and dark-stained pynotic

nuclei, were observed in the islet cells, especially located in the central part. The morphometric measurements also supported these observations, where the islet diameter/area were significantly decreased in the STZ-induced diabetic rats. These findings were in agreement with previous studies that used the same dose of STZ to induce DM (Ahmed et al. 2010; Abdel Aziz et al., 2013; Yang and Kang 2018). STZ leads to alkylation of the DNA, after being taken up into the β-cell via a glucose transporter (GLUT 2). Poly ADP-ribosylation, as a result of DNA damage, causes NAD+ and adenosine triphosphate (ATP) depletion in the β-cell. Increased ATP dephosphorylation provides a substrate for xanthine oxidase, which demonstrates high activity in this cell. Superoxide radicals then develop and following that, hydrogen peroxide and hydroxyl radicals appear, and the cytotoxic action of STZ is demonstrated by the generation of reactive oxygen species. Finally, the β-cell are degenerated and necrosis occurs (Szkudelski, 2001). In the DM + Aa group, the negative impacts induced by STZ were improved, where the histopathological changes observed were lower and the islet diameter/area were significantly increased when compared to the DM group. Supporting those findings, the immuno-histochemical staining showed that decreased numbers of β-cells by STZ-inducement were reconstructed and the β-cell index significantly increased to the levels observed in the DM + Gli group. C-peptide, which is an important protein for insulin synthesis, connects to the α- and β-chains of insulin in proinsulin. This molecule possesses an insulin-mimicking effect via the activation of insulin receptors and increases in the uptake of amino acid and the synthesis of glycogen. Insulin action is promoted by C-peptide at low levels of hormone and inhibited at high levels, which suggests an effect that regulates insulin signal transduction (Grunberger and Sima, 2004; Cersosimo et al., 2014). Additionally, in this study, insulin, as well as C-peptide deficiencies, in diabetic rats, caused by the damage in β-cells as a consequence of the impact of STZ were improved by the administration of ethanolic Aa extract. It has been reported that plant-derived compounds display their mechanisms of action by increasing the size and number of cells in the islet of Langerhans, protecting β-cells from destruction, regenerating and/or repairing the β-cells, enhancing insulin secretion, preventing β-cell apoptosis, preventing oxidative stress, and modulating β-cell differentiation and proliferation (Jarald et al., 2008; Hosseini et al., 2015; Oh et al., 2015; Uyar et al., 2017; Yaman et al., 2017). The flavonoids and phenolic compounds derived from plants are well-known to exert antidiabetic and antioxidant activities (Li et al., 2014; Dra et al., 2018; Sekiou et al., 2019). Phenolic compounds, such as quercetin and rutin, protect β-cells from STZ-induced oxidative stress, elevate blood insulin and C-peptide levels, and participate in the regeneration of β-cells (Ahmed et al., 2010; Coskun et al., 2005; Yang and Kang, 2018). On the other hand, flavonoids, such as quercetin and (−)epicatechin, may, at least in part, cause an insulin release changing Ca^{2+} metabolism in isolated islet cells (Hi and Howell, 1985). The phytochemical screening of methanolic Aa extract from showed rich phenol and flavonoid contents of its leaves and inflorescence ranging from 149–1657 mg of gallic acid equivalent per 100 g dry weight (dw) and 59–264 mg of quercetin equivalent per 100 g dw, and it has been shown to possess antioxidant activity (Varasteh-Kojourian et al., 2015). In an another study, 8 phenolic compounds, including quercetin 3-β-D-glucoside, ferulic acid, rutin, quercetin, kaempferol, myricetin, luteolin, caffeic acid, were identified in the aqueous and ethanolic extracts of the flowering aerial parts of Aa, and the major phenolic compound was detected as quercetin 3-β-D-glucoside, and it was reported that the extract from Aa showed radical scavenging activity (Hammad et al., 2013). The main components of Aa essential oil were determined as borneol (4.4%), chrysanthenone (8.2%), camphor (8.8%), eucalyptol (13.0%), and piperitone (34.9%), which comprise 68.3% of the part (Sökmen et al., 2004). Aa essential oil has been shown to be hepatoprotective and it has shown antioxidant activity on CCl_4-induced liver fibrosis in rats (Al Said et al. 2016). Abd-Alla et al. (2016) isolated 3 sesquiterpene lactones and 4 derivatives of 3-methoxy flavones from extract of the aerial parts of Aa, and the extract protected rats against ethanol-induced gastric ulcers and inhibited α-amylase activity that resulted in a postprandial hyperglycaemia lowering effect. In accordance with in this study, the other members of the genus *Achillea* L. have been shown to possess antidiabetic and antioxidant activities. For example, extracts from aerial parts of *A. fragrantissima*, containing polyphenols (gallic acid quivalent) and flavonoids (rutin equivalents), displayed therapeutic potential in the amelioration of a high-fat diet and diabetic rats by exhibiting very promising antidiabetic, hypolipidemic, aninflammatory, and antioxidant activities (Abd El-Fattah et al. 2018). In another study, the methanolic extract of *A. lingustica*, constituting mainly the triterpene moretenol, showed radical scavenging and antidiabetic activities (Conforti et al. 2005). Thus, the mitigating and secretory effect of Aa might be attributed to its phenolic and flavonoid content, showing its free radical scavenging and protective activity, and regenerative role on β-cells in the Langerhans islets.

CONCLUSION

The data obtained in this study showed that i.p. administration of STZ caused histopathological alterations, decreases in the β-cell number in the islets,

and decreased levels of blood insulin and C-peptide levels in rats. However, the administration of ethanolic Aa extract at a dose of 400 $mg^{-kg\ bw}$ attenuated the pathological alterations and partly regenerated the β-cell number comparing Gli-treated rats, leading to insulin and C-peptide secretion. Hence, it can be concluded that the plant has a islet-protective potential, but more research is necessary to determine the exact mechanism of action of Aa in a protective role on the islet architecture and function in STZ-induced DM.

Acknowledgments and Funding

This study was partially supported by the Scientific Research Projects Coordination Unit of Van Yuzuncu Yil University under project numbers TYL-2019-865 and FAP-2019-8503. Thanks are given to Dr. Süleyman Mesut Pınar for his helpful taxonomical identification of the plant material.

Conflict of Interest

The authors declare that they do not have any competition and any conflicts of interest.

Author Contributions

AD and HCH conceived the research. AD and HCH contributed in preparation of the plant extract, animal handling and treatments. BK performed the histological and immunohistochemical works. BK and AD performed the biochemical analyses, analyzed the data and interpreted the results. BK drafted, edited and finalized the manuscript.

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