Research Article

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The effect of *Diplotaenia turcica* root extract in streptozotocin-induced diabetic rats

Diplotaenia turcica kök ekstraktının diyabetik ratlar üzerine etkisi

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Abstract

Background: *Diplotaenia turcica* has been used traditionally to diabetes treatment. In this study, the effects of *D. turcica* root extract (DT) on diabetes mellitus induced by streptozotocin (STZ) were investigated.
Materials and methods: In this study, 78 male rats were used, rats were divided into 9 groups randomly. In diabetic groups, STZ was given a single dose of 45 mg/kg by intraperitoneally. DT (50, 100 and 200 mg/kg) and glibenclamide (5 mg/kg) were given by orally. Blood and pancreas tissue samples were taken for biochemical and pathological tests.
Results: It was found that glucose levels decreased, and insulin levels increased in the treatment groups compared with the diabetes group. In addition, only in 200 mg/kg DT dose group was found to decrease HbA1c levels. Pancreatic tissue analysis showed that MDA levels decreased and GSH levels and CAT, SOD, GSH-Px and GSH-R activities increased in diabetic rats treated with DT. Histopathological and immunohistochemical examinations of the pancreas showed significant improvements in the treatment with DT.
Conclusion: These results clearly show the antioxidant property of DT. The findings of this study showed that increased doses of DT may have a therapeutic effect on STZ-induced pancreatic damage.

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Keywords: Antioxidant; Diabetes mellitus; *Diplotaenia turcica*; Histopathology; Immunohistochemistry; Lipid peroxidation; Pancreas.

Öz

Amaç: *Diplotaenia turcica* geleneksel olarak diyabet tedavisi için kullanılmaktadır. Bu çalışmada, *Diplotaenia turcica* kökü ekstraktının (DT), streptozotosin (STZ) indüklenerek oluşturulan diabetes mellitus'taki bazı etkileri incelenmiştir.
Gereç ve Yöntem: Bu çalışmada toplam 78 adet erkek rat kullanıldı, ratlar rastgele 9 gruba ayrıldı. Diyabetik gruplarda STZ tek doz, 45 mg/kg dozunda inraperitonel olarak uygulandı. DT (50, 100 ve 200 mg/kg) ve glibenklamit (5 mg/kg) oral yolla uygulandı. Biyokimyasal ve patolojik değerlendirmeler için kan ve pankreas doku örnekleri alındı.
Bulgular: Diyabet grubuna kıyasla tedavi gruplarında glukoz seviyesinin azaldığı ve insülin seviyesinin arttığı tespit edildi. Ayrıca, yalnızca 200 mg/kg DT dozu uygulanan grupta, HbA1c seviyesinin azaldığı belirlendi. Pankreas dokusu analizlerinde, DT uygulanan diyabetic ratlarda, MDA seviyesinin düştüğü ve GSH seviyesi ile CAT, SOD, GSH-Px, GSH-R aktivitelerinin arttığı görüldü. Histopatolojik ve immunohistokimyasal incelemeler, DT'nin pankreas hasarını önemli ölçüde iyileştirdiğini göstermiştir.
Sonuç: Bu sonuçlar açıkça DT'nin antioksidan özelliğinin olduğunu göstermektedir. Elde edilen bulgular, artan DT dozlarının STZ'nin neden olduğu pankreas hasarı üzerinde terapötik bir etkiye sahip olabileceğini göstermiştir.

Anahtar kelimeler: Antioksidan; Diabetes mellitus; *Diplotaenia turcica*; Histopatoloji; İmmunohistokimya; Lipit peroksidasyon; Pankreas.

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Introduction

Diabetes mellitus (DM) is a metabolic disease that develops due to the irregularities in the carbohydrate, fat and protein metabolisms stemming from disorders of secretion or from the effect of insulin hormone, or both. In diabetes and other metabolic diseases, it has been observed that the oxidative stress induced by the increase in free radical levels and the decrease in antioxidant defense level is effective [1]. Likewise, the cell and tissue damage have also been observed to increase with the oxidative stress in diabetes, as well [2].

It is important to control different metabolic disorders in order to prevent and control the complications based on diabetes [3]. In case of diabetes in particular, the amounts of antioxidants, which scavenge free radicals directly or prevent them from turning into toxic products, change [1].

The extrinsic antioxidants have a protective effect against the oxidative damages of the free radicals as well as play an important role in the healing process of the disease [4, 5]. Positive effects of many plants, which have antioxidant properties and are used in the traditional treatment of diabetes, have been supported in several scientific studies [3, 6]. Both preclinical and clinical studies have reported that about 800 medicinal herbs with potential antidiabetic activity are effective due to their bioactive compounds such as alkaloids, terpenoids, and flavonoids [7].

Diplotaenia turcica (DT) is a member of family Apiaceae (Umbelliferae) [8]. A number of scientific studies have revealed that some species from the Apiaceae family are effective for treatment of diabetes [9–12] DT is used as a traditional treatment as well as an ingredient in many dairy products such as herby cheese. It is also used to protect people from the bites of venomous animals like snake etc. Its root is used to treat rheumatism, diabetes, and blood pressure for many centuries [13, 14]. In our previous study, LD_{50} value of DT was found to be higher than 5000 mg/kg in terms of acute toxicity. No mortality and adverse effects were recorded in rats treated orally at 250, 500 and 1000 mg/kg for 28 days. No significant difference was observed between control and treatment groups in terms of mean baseline and final body weight, organ weight, and hematological parameters. The cholesterol, triglycerides and LDL levels alongside glucose level (250 mg/kg/28 days) significantly decreased in all treatment groups. Total phenolic content (72.65 ± 2.20 μgPEs/mg) of DT was higher than its flavonoid content (8.30 ± 0.61 μgQEs/mg). The antioxidant properties of many plant extracts have been attributed to their phenolic and flavonoid content [10]. DT exhibits more moderate activity than butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) using standard CUPRAC at 100 μg/mL.

As a result of the reviews, no study investigating the effect of DT plant on diabetes was found. Therefore, this study set out to assess the effect of the hydroalcoholic root extract of DT on lipid peroxidation, antioxidants, and immunohistochemistry in the pancreatic tissue of streptozotocin (STZ) induced diabetic rats.

Materials and method

Plant material

DT was collected in Hakkari, Turkey, and identified in May-June. Voucher specimen (VANF 32858) is kept in the Herbarium of Van Yüzüncü Yıl University.

Preparation of the extract

The roots of the plant were dried up and pulverized by using an electrical mill. A 100 g powder sample was added into 1000 mL of alcohol. First, 96% ethanol was utilized and whereupon the solution was filtered 24 h later. Then, 70% ethanol was added into the dry matters and the solution was filtered 24 h later. Afterwards, both filtered solutions were mixed and evaporated repeatedly using a rotary evaporator (50°C, 70 rpm). Concentrated extracts were lyophilized to yield 6% w/w dry extract and then were stored at –20°C. In order to prepare the injected extract, this powder was dissolved in a specific volume of normal saline [15].

Animals

Female Wistar albino rats (200–250 g) were chosen. The animals were allowed to eat and drink water. They were also treated under standard laboratory conditions, including a 12-h light-dark cycle and room temperature of 22 ± 2°C. They were acquired from the Experimental Research Laboratory of Yüzüncü Yıl University's Faculty of Medicine. The protocol of the experiment was confirmed by the same university's Ethics Commission for Animal Use (2016/05).

Experimental protocol

In the 28-day experimental study, 78 male Wistar albino rats were divided into 6 groups as follows:

(i) Group I (n = 7): Control group eating a normal diet

(ii) Group II (n = 10): Diabetic rats (treated intraperitoneally with STZ of 45 mg/kg dissolved in 0.1 M citrate buffer) [16]
(iii) Group IIIa (n = 7): Control group of normal rats treated with DT at a dose of 50 mg/kg/day
(iv) Group IIIb (n = 10): Diabetic rats treated with DT at a dose of 50 mg/kg/day
(v) Group IVa (n = 7): Control group of normal rats treated with DT at a dose of 100 mg/kg/day
(vi) Group IVb (n = 10): Diabetic rats treated with DT at a dose of 100 mg/kg/day
(vii) Group Va (n = 7): Control group of normal rats treated with DT at a dose of 200 mg/kg/day
(viii) Group Vb (n = 10): Diabetic rats treated with DT at a dose of 200 mg/ kg/day
(ix) Group VI (n = 10): Rats treated with glibenclamide at a dose of 5 mg/kg [17].

Diabetic rats having serum glucose levels >200 mg/dL, 72 h after administration of STZ were included in the study [16]. The doses of the plant DT were determined in parallel with the dose, which was effective in lowering the glucose level [8]. The DT and glibenclamide were administered via an oral gavage.

Biochemical examination

Four weeks later, the hearts of the rats were directly cannulated after administering 75 mg/kg ketamine (ip) and their blood samples were placed both in the glass serum tubes and EDTA tubes. The blood samples were then centrifuged at +4°C and 3000 rpm for 10 min. The concentrations of glucose, triglyceride, cholesterol and HDL cholesterol were determined in an automated analyzer (ArchitecCi 16000) using the Abbott commercial kit. The insulin levels of the samples were measured using ELISA (Biotek ELx800) and a commercial kit (Eastbiopharm no: CK-E30620). LDL cholesterol levels were calculated according to Friedewald formula [18].

$$\text{LDL cholesterol} = \text{cholesterol} - (\text{HDL cholesterol} + \text{TG} / 5)$$

The amount of HbA1c in the whole blood was determined on the same day using an auto-analyzer (Roche Cobas Integra 400 plus).

The pancreatic tissues of the rats were sacrificed by exsanguination and removed. Half of the pancreatic tissues were separated for the biochemical study, whereas the other half was separated for the histopathological examination. The extracted pancreatic tissues were homogenized in 10 mL PBS on ice using a mechanical homogenizer (ISOLAB no: 621.11.001) and samples were centrifuged at $1000 \times g$ for 10 min at 4°C to remove large insoluble particles. MDA (SunRed no: 201-11-0157), GSH (Glutathione) (SunRed no: 201-11-5431), CAT (Catalase) (SunRed no: 201-11-5106), SOD (Superoxide dismutase) (SunRed no: 201-11-0169), GSH-Px (SunRed no: 201-11-5104), and GSH (Glutathione)-R (SunRed no: 201-11-5111) analyses were conducted using the related commercial kits in the ELISA device (Biotek ELx800).

The histopathological analysis of pancreatic tissue

The pancreas tissue samples, which were taken for histopathological analysis, were fixed in 10% formalin solution for 48 h and then were washed under running water for 8 h. They were treated with alcohol (70°, 80°, 90°, 96°, and 100°) and a series of xylene during the routine tissue control period and then were blocked in paraffin. The samples were prepared on the slides by taking 4-μm sections from each block. They were prepared for the histopathological analysis and stained with hematoxylin–eosin (HE) staining. The relevant areas were photographed and analyzed using a light microscope (Olympus BX51 optical microscope and Olympus DP25 digital camera, Japan). The tissues were rated as negative (–), slight (+), moderate (++), or severe (+++) according to the histopathological findings.

The immunohistochemical analysis of pancreatic tissue

All of the sections were taken onto the adhesive (poly-L-lysin) microscope slide for immunoperoxidase examination and passed through xylol and alcohol series. The sections were washed with PBS, whereupon endogenic peroxidase inactivation was then provided by keeping them in H_2O_2 of 3% for 10 min. In order to reveal the antigen in the tissues, it was treated with antigen retrieval solution in a microwave oven at 2×5 min 500 W, and then left to cool. The tissues were incubated with insulin (Catalogue no: sc-8033, Santa Cruz, USA) for 60 min at 37°C according to the immunohistochemistry kit procedure (AbcamHRP/DAB Detection IHC kit). 3,3′-Diaminobenzidine (DAB) was used as chromogen. The ground staining was performed with hematoxylin. The sections were evaluated as none (–), mild (+), moderate (++), and severe (+++) based on their immunopositivity.

Statistical analysis

The data were expressed as mean ± SD and analyzed using IBM SPSS V 23.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis was carried out by one-way analysis of variance (ANOVA), which was followed by Duncan's multiple comparison test. The value of $p = 0.05$ was accepted as statistically significant.

Results

The diagnosis of diabetes was confirmed with the increased serum glucose level. At the end of the 28-day trial, the serum glucose level was measured as 166.86 ± 0.36 mg/dL in Group I and 654.60 ± 18.80 mg/dL in Group II, which consisted of STZ-induced diabetic rats. It was observed that the glucose levels of the Groups IIIa, IVa, and Va were close to those of the control group. The glucose levels of the Groups IIIb, IVb, and Vb were higher than Group I but lower than Group II. Moreover, there was a decrease parallel to the increase in DT dosage.

The serum insulin level of the Group I was 34.88 ± 0.36 mIU/L. The serum insulin level of the STZ-induced rats (Group II) was lower than the Group I ($p < 0.05$). However, although this value was lower in the Groups IIIb, IVb, and Vb (diabetic groups treated with DT) than the Group I, they was significantly higher than the Group II. The insulin level was higher in the Groups IIIa, IVa, and Va (groups in which diabetes was not induced and which were treated with DT) than the Group II; likewise, their values were close to the Group I.

The HbA1c levels of the Group II were significantly higher than the control group ($p < 0.05$). The HbA1c levels of the Groups IIIb and IVb were close to the level of Group II, and a significant decrease was observed in the HbA1c levels of the Group Vb ($p < 0.05$). The HbA1c values of these three groups were significantly higher than the Group I (Table 1).

A significant increase was observed in the triglyceride (TG) and LDL cholesterol levels in Group II compared to Group I. No significant change was observed in the increased TG and LDL cholesterol level in the Groups IIIb, IVb, and Vb (the diabetic groups treated with DT). There was no significant difference between the treatment groups in terms of cholesterol and HDL cholesterol levels (Table 1).

As seen from Table 2, the activities of pancreatic antioxidants such as GSH, CAT, SOD, GSH-Px and GSH-R reduced significantly in diabetic rats (Group II) compared to the control rats (Group I) ($p < 0.05$). Oral DT and glibenclamide treatment significantly improved the pancreatic antioxidants in the diabetic rats ($p < 0.05$). In particular, the level of improvement significantly increased in the group receiving 200 mg/kg DT compared to the oral glibenclamide. The MDA level in the pancreatic tissues of diabetic rats had also significantly increased compared to the control. These levels significantly decreased in the diabetic groups as a result of DT administration (in doses of 50, 100, and 200 mg/kg/day) ($p < 0.05$).

Table 1: The effect of hydroalcoholic extract of *Diplotaenia turcica* on glucose, insulin, HbA1c, TG, cholesterol, HDL cholesterol and LDL cholesterol in control and treatment groups.

Group	Serum glucose mg/dL X±Sx	Serum insulin (mIU/L) X±Sx	HbA1c (%) X±Sx	TG (mg/dL) X±Sx	Cholesterol (mg/dL) X±Sx	HDL cholesterol (mg/dL) X±Sx	LDL cholesterol (mg/dL) X±Sx
Group I	166.86 ± 7.15^{d}	34.88 ± 0.36^{a}	3.92 ± 0.14^{d}	43.29 ± 1.98^{b}	46.43 ± 1.89	32.87 ± 1.39	4.90 ± 1.23^{b}
Group II	654.60 ± 18.60^{a}	12.99 ± 0.67^{e}	6.41 ± 0.03^{ab}	69.86 ± 2.14^{a}	55.14 ± 1.91	30.71 ± 1.41	10.49 ± 1.26^{a}
Group IIIa	148.50 ± 10.89^{d}	33.60 ± 0.83^{a}	3.99 ± 0.05^{d}	44.43 ± 5.08^{b}	50.00 ± 2.54	35.90 ± 2.92	5.21 ± 1.07^{b}
Group IIIb	538.40 ± 12.74^{b}	17.91 ± 0.95^{d}	6.60 ± 0.13^{a}	51.86 ± 8.29^{ab}	48.43 ± 4.21	30.58 ± 1.64	7.50 ± 1.43^{ab}
Group IVa	157.14 ± 6.28^{d}	34.60 ± 0.51^{a}	3.95 ± 0.04^{d}	45.44 ± 4.88^{b}	47.71 ± 1.85	33.71 ± 0.99	4.91 ± 2.38^{b}
Group IVb	468.50 ± 4.06^{c}	21.50 ± 0.95^{c}	6.30 ± 0.03^{b}	52.86 ± 9.72^{ab}	47.14 ± 5.23	30.07 ± 2.96	6.50 ± 1.04^{ab}
Group Va	164.29 ± 5.58^{d}	34.73 ± 0.51^{a}	3.96 ± 0.05^{d}	46.29 ± 3.63^{b}	47.26 ± 4.47	33.07 ± 2.62	5.11 ± 1.66^{b}
Group Vb	461.20 ± 6.93^{c}	24.86 ± 0.84^{b}	6.04 ± 0.12^{c}	52.43 ± 8.92^{ab}	47.29 ± 4.63	30.26 ± 2.90	6.54 ± 1.85^{ab}
Group VI	532.30 ± 15.13^{b}	20.68 ± 0.54^{c}	6.58 ± 0.12^{a}	50.86 ± 4.56^{ab}	45.91 ± 1.96	29.94 ± 1.01	5.60 ± 0.97^{b}

Group I: Control (vehicle treated), Group II: Diabetic control (SZT, 45 mg/kg, ip, once weekly), Group IIIa: DT 50 mg/kg/day, Group IIIb: Diabetic + DT 50 mg/kg/day, Group IVa: DT 100 mg/kg/day, Group IVb: Diabetic + DT 100 mg/kg/day, Group Va: DT 200 mg/kg/day, Group Vb: Diabetic + DT 200 mg/kg/day, Group VI: Diabetic + glibenclamide 5 mg/kg/day.
Values are expressed as mean ± SD for seven animals. Values not sharing common superscript letters (a–e) in the same column differ significantly at $p < 0.05$.

Table 2: The effect of the hydroalcoholic extract of *Diplotaenia turcica* on MDA and GSH levels and CAT, SOD, GSH-Px, and GSH-R activities in the pancreatic tissues of control and treatment groups.

Group	MDA (nmol/mL) X±Sx	GSH (mg/L) X±Sx	CAT (ng/mL) X±Sx	SOD (IU/mL) X±Sx	GSH-Px (ng/mL) X±Sx	GSH-R (ng/mL) X±Sx
Group I	2.97 ± 0.05^{de}	344.90 ± 8.33^{e}	13.94 ± 0.16^{a}	130.00 ± 1.83^{c}	41.50 ± 0.29^{bc}	24.80 ± 0.78^{cd}
Group II	7.93 ± 0.26^{a}	211.94 ± 4.33^{g}	4.38 ± 0.13^{e}	54.77 ± 2.30^{g}	19.78 ± 0.82^{f}	13.48 ± 0.49^{g}
Group IIIa	2.84 ± 0.15^{e}	349.47 ± 12.44^{e}	14.04 ± 0.37^{a}	136.68 ± 8.75^{bc}	43.45 ± 1.36^{ab}	26.15 ± 0.94^{bc}
Group IIIb	6.25 ± 0.13^{b}	421.30 ± 3.82^{bc}	5.99 ± 0.42^{d}	79.66 ± 1.35^{f}	36.78 ± 0.62^{d}	20.08 ± 0.31^{f}
Group IVa	2.80 ± 0.12^{e}	384.66 ± 5.38^{d}	14.10 ± 0.52^{a}	142.68 ± 3.04^{ab}	44.49 ± 0.53^{a}	27.84 ± 0.56^{ab}
Group IVb	4.13 ± 0.25^{c}	434.46 ± 8.61^{b}	9.01 ± 0.17^{c}	112.67 ± 1.45^{d}	38.88 ± 0.54^{cd}	22.51 ± 0.62^{e}
Group Va	2.74 ± 0.06^{e}	410.30 ± 8.04^{c}	14.20 ± 0.48^{a}	149.22 ± 1.43^{a}	44.88 ± 0.56^{a}	28.43 ± 0.77^{a}
Group Vb	3.46 ± 0.17^{d}	460.14 ± 3.00^{a}	11.11 ± 0.22^{b}	127.55 ± 2.21^{c}	39.25 ± 1.19^{cd}	23.29 ± 0.33^{de}
Group VI	4.48 ± 0.17^{c}	300.55 ± 6.30^{f}	8.10 ± 0.31^{c}	100.50 ± 2.19^{e}	29.15 ± 1.65^{e}	19.73 ± 0.65^{f}

Group I: Control (vehicle treated), Group II: Diabetic control (SZT, 45 mg/kg, ip, once weekly), Group IIIa: DT 50 mg/kg/day, Group IIIb: Diabetic + DT 50 mg/kg/day, Group IVa: DT 100 mg/kg/day, Group IVb: Diabetic + DT 100 mg/kg/day, Group Va: DT 200 mg/kg/day, Group Vb: Diabetic + DT 200 mg/kg/day, Group VI: Diabetic + glibenclamide 5 mg/kg/day.
Values are expressed as mean ± SD for seven animals. Values not sharing common superscript letters (a–g) in the same column differ significantly at $p < 0.05$.

The pancreatic histopathology

Figure 1 and Table 3 show the histopathological structure of the pancreatic tissue, and its analysis, effectively. It was observed that the pancreatic β-cells of the diabetic rats were completely damaged, due to streptozotocin induction compared to the control group (Table 3). It was revealed that the atrophy of the langerhans islets, degeneration, and necrosis in the β-cells decreased significantly and the pancreatic structure reformed in the diabetic rats after the DT and glibenclamide administrations. DT did not cause damage in the pancreatic β-cells of the healthy rats that were treated with DT.

Immunohistochemical assessment

It was observed that Group I's pancreatic Langerhans islets had insulin immunoreactivity severity (+++), whereas it was slight (+) in the Group II. In diabetic rats, insulin immunoreactivity of 50 and 100 mg/kg DT groups was (+) and moderate (++), respectively. Insulin immunoreactivity was severe in diabetic rat groups treated with glibenclamide and 200 mg/kg DT (Groups VI and Vb). The insulin immunoreactivity in the pancreatic Langerhans islets of the healthy rats, who were treated with 50, 100 and 200 mg/kg DT, was severe (+++) (Figure 2, Table 4).

Discussion

Diabetes mellitus (DM) is a metabolic disease that causes uncontrollable complications in many tissues and organs such as liver, kidney, and the pancreas [4]. Hyperglycemia, induced by streptozotocin (STZ), is described as a beneficial experimental model in order to examine how antidiabetic agents affect diabetes. STZ is used for experimental diabetes, has toxic effects on pancreatic beta cells, and causes oxidant effects and the formation of nitric acid together with DNA damage [19, 20].

The oxidative stress developing after the balance between the oxidants and antioxidants becomes impaired in favor of the oxidants is known to play an important role in the etiology and progression of diabetes [21].

Being involved in the sulfonylureas group of oral antidiabetics, glibenclamide is used as a reference drug in the chemically induced experimental diabetes studies. The antihyperglycemic effect of sulfonylureas occurs through increased insulin secretion from pancreatic beta cells [22].

After pointing out that free radicals play a role in the formation of diabetes, it has been suggested that the antioxidants blocking the radical formation and the plants with antioxidant properties could be used to treat the disease and support this treatment. However, chemical drugs are expensive and have many side effects; therefore, the alternative and complementary treatments have been begun to be used [4]. For this purpose, the herbal therapy methods have been rapidly involved in the studies as an alternative therapy for diabetes and its complications. It has been proven that many plants used for traditional treatment of diabetes have positive effects [3, 6].

The experimental methods have shown the effects of some species of the Apiaceae family, which are used to treat diabetes [10, 12, 15, 23]. DT, which belongs to Apiaceae family [24], has been reported to be used for

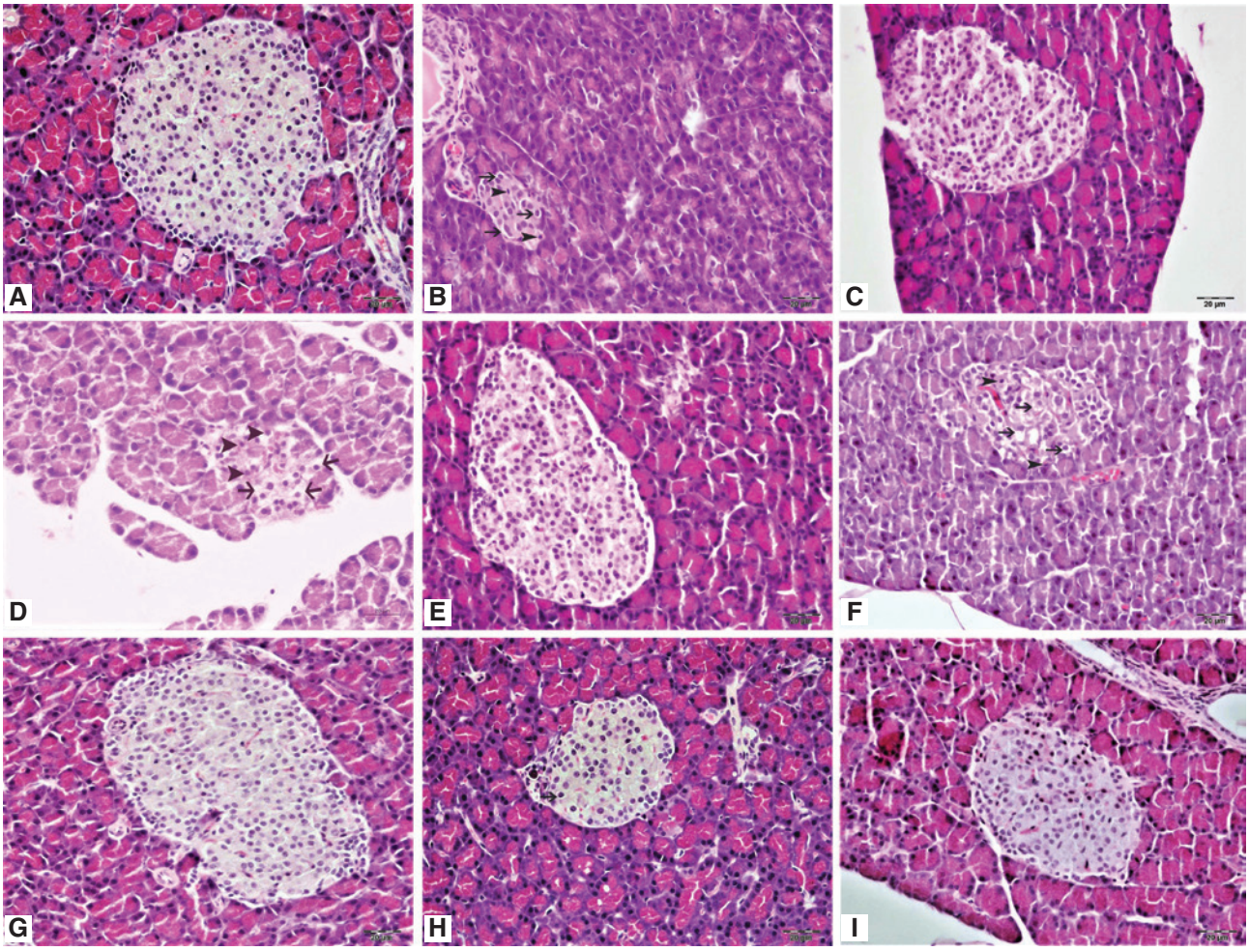

Figure 1: Histopathology of the pancreatic tissue of control and treatment groups of hydroalcoholic extract of *Diplotaenia turcica*. (A) Group I: Control (vehicle treated), normal histological structure, (B) Group II: Diabetic control (STZ, 45 mg/kg, ip, once weekly), atrophy in the islet of langerhans, degeneration and necrosis in islet cells, (C) Group IIIa: DT 50 mg/kg/day, normal histological structure, (D) Group IIIb: Diabetic + DT 50 mg/kg/day, atrophy in the islet of langerhans, degeneration and necrosis in islet cells, (E) Group IVa: DT 100 mg/kg/day, normal histological structure, (F) Group IVb: Diabetic + DT 100 mg/kg/day, very mild atrophy in langerhans islet, degeneration in islet cells and small number of necrosis, (G) Group Va: DT 200 mg/kg/day, normal histological structure, (H) Group Vb: Diabetic + DT 200 mg/kg/day, mild atrophy in langerhans islets, small number of degeneration in islet cells, (I) Group VI: Diabetic + glibenclamide 5 mg/kg/day, mild atrophy in langerhans islet, degeneration in islet cells and small number of necrosis, (→) hydropic degeneration, (►) necrosis, H&E, Bar: 20 μm.

Table 3: Rating the histopathology effect of hydroalcoholic extract of *Diplotaenia turcica* on pancreatic tissue of control and treatment groups.

	Group 1	Group 2	Group IIIa	Group IIIb	Group IVa	Group IVb	Group Va	Group Vb	Group VI
Atrophy in the islets of Langerhans	–	+++	–	++	–	+	–	–	–
Hydropic degeneration in β-cells	–	+++	–	+++	–	++	–	+	++
Necrosis in β-cells	–	++	–	++	–	+	–	–	+

The sections examined in the light microscope were evaluated as negative (–), slight (+), moderate (++) and severe (+++) according to the lesions.

traditional treatment of diabetes in the region of Hakkari [8, 13].

The studies featuring that *Prangos ferulacea* [25], *Cuminum cyminum* [10], *Petroselinum sativum* [12], and Coccinia *grandis* [22] plant extracts of the Apiaceae family are used, as a result of the STZ injection reported that the serum insulin level decreases and the glucose and HbA1c level increase in the diabetic rats, compared to the

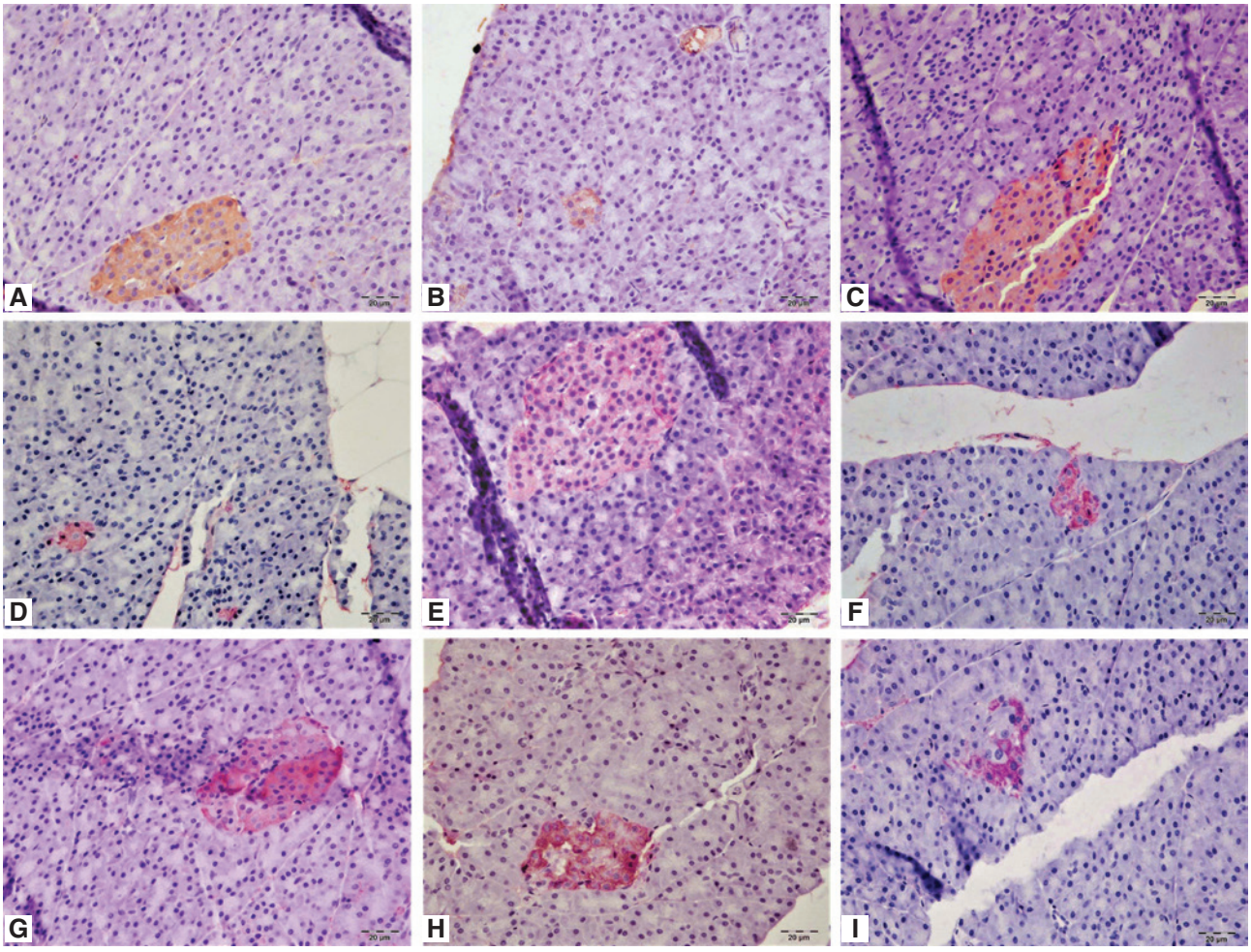

Figure 2: Insulin immunoreactivity of the effect of the hydroalcoholic extract of *Diplotaenia turcica* on pancreatic langerhans islets of control and treatment groups.
(A) Group I: Control (vehicle treated), severely insulin expression, (B) Group II: Diabetic control (SZT, 45 mg/kg, ip, once weekly) slightly insulin expression, (C) Group IIIa: DT 50 mg/kg/day, severely insulin expression, (D) Group IIIb: Diabetic + DT 50 mg/kg/day, slightly insulin expression, (E) Group IVa: DT 100 mg/kg/day, severely insulin expression, (F) Group IVb: Diabetic + DT 100 mg/kg/day, moderately insulin expression, (G) Group Va: DT 200 mg/kg/day, (H) Group Vb: Diabetic + DT 200 mg/kg/day, severely insulin expression (I) Group VI: Diabetic + glibenclamide 5 mg/kg/day, severely insulin expression, IHC-P, Bar: 20 µm.

Table 4: Rating the scoring of insulin immunoreactivity effect of the hydroalcoholic extract of *Diplotaenia turcica* on pancreatic langerhans islets of control and treatment groups.

Group	1. Rat	2. Rat	3. Rat	4. Rat	5. Rat	6. Rat	7. Rat
Group I	+++	+++	+++	+++	+++	++	++
Group II	+	+	+	–	+	–	+
Group IIIa	++	+++	+++	+++	+++	++	+++
Group IIIb	+	++	++	++	+	+	++
Group IVa	+++	++	+++	+++	+++	+++	+++
Group IVb	++	++	+	++	++	++	+
Group Va	+++	+++	+++	++	+++	+++	+++
Group Vb	+++	++	++	+++	+++	++	+++
Group VI	++	++	++	++	++	++	+

There is negative (–), slight (+), moderate (++) and severe (+++) insulin immunoreactivity of β-cells. Group I: Control (vehicle treated), Group II: STZ-induced diabetic (45 mg/kg, ip, once weekly), Group IIIa: DT, 50 mg/kg/day, Group IIIb STZ-induced diabetic + DT, 50 mg/kg/day, Group IVa: DT, 100 mg/kg/day, Group IVb: STZ-induced diabetic + DT, 100 mg/kg/day, Group Va: DT, 200 mg/kg/day, Group Vb: STZ-induced diabetic + DT, 200 mg/kg/day, Group VI: STZ-induced diabetic + glibenclamide, 5 mg/kg/day.

control group, due to the damage in the beta cells and the serum insulin levels increase and the glucose and HbA1c levels decrease after the administration of plant extracts. However, other studies have revealed that administrations of the *Petroselinum crispum* [9], *Ferula assafoetida* [11] and Eryngium *carlinae* [26] plant extracts of the Apiaceae family do not affect glucose levels.

The present study revealed that the HbA1c and blood glucose levels increased and the insulin level decreased following the 45 mg/kg (ip) administration of STZ. This result showed that the insulin synthesis decreased due to the damage induced by STZ in pancreatic beta cells and, as a result, the glucose level in the blood increased. These results are compatible with the aforementioned studies. In the diabetic rats treated with STZ, an increase in DT dose was found to improve the glucose and insulin values significantly.

Lipid profile abnormalities are observed in approximately 40% of the diabetic cases, and therefore are one of the most common symptoms of diabetes [27]. The studies investigating the effect of diabetes on the lipid-structured compounds in the blood have shown that the serum triglyceride, cholesterol, and LDL cholesterol levels increase, whereas HDL cholesterol levels decrease [18]. Conversely, Karaca et al. reported that the triglyceride and cholesterol levels decreased in diabetes [28]. Different results have been obtained in studies examining the effects of different species of the Apiaceae family on serum lipid profile in the diabetes-induced rats [11, 26, 29]. In the present study, a significant increase was observed in the triglyceride and LDL cholesterol and no significant change was determined in the cholesterol and HDL-cholesterol levels in diabetic group. Although the TG and LGL-cholesterol levels decreased, no statistical difference was found after the 28-day DT and glibenclamide administration.

Oxidative stress develops in the STZ-induced diabetic rats as the formation speed of the free radicals increases, thus rendering antioxidant defense inadequate [12]. In this case, free oxygen radicals cause lipid peroxidation and damage cell functions. Malondialdehyde (MDA) is one of the products forming as a result of the lipid peroxidation and causes the cell dysfunction due to its negative effects such as affecting the cell membrane lipids and changing the enzyme activities. In addition, MDA level is used as an important parameter in order to determine the amount of oxidative stress [30].

Studies on STZ-induced diabetes reported that the pancreatic MDA level increased significantly compared with the control group [1, 18, 29, 31–35]. It was determined that the increased MDA amount in the pancreatic tissue and plasma reduced significantly by the administration of the plant extracts of the Apiaceae family [10, 12]. Similarly, MDA level in the pancreatic tissue of the untreated diabetic rats was observed to increase significantly. The fact that MDA level decreased when DT was administered to diabetic rats was significant. Likewise, the results of the group in which 200 mg/kg DT was administered were very close to the control group. This was thought to be associated with the fact that DT inhibited the lipid peroxidation due to its antioxidant characteristics in the defense against the oxidative damage induced by diabetes [36].

Antioxidant enzymes are used to render free radicals ineffective or less harmful. Thus, they protect the organs and membranes against oxidative damage [1]. GSH is an intracellular, non-enzymatic antioxidant substance. They prevent oxidative damage from occurring due to oxidative stress by reacting the peroxides and free radicals directly. GSH acts as both a substrate for antioxidant enzymes and a radical scavenger while counteracting the damage of radicals [37].

In the literature reviews, different results have been found to be associated with antioxidant levels and antioxidant activity in the pancreatic tissue of STZ-induced rats. In one study, pancreatic SOD and CAT enzyme activities were reported to increase compared to the control group [36]; whereas, another study revealed no change in SOD activity [38]. Unlike, other studies reported that GSH levels as well as SOD, CAT, GSH-Px [1, 29, 35] and GSH-R [1, 31, 33] activities decreased significantly. However, it was determined that the GSH level and SOD and CAT enzyme activities [10] and the plasma total antioxidant capacity (TAC) [12] of the pancreatic tissue, which was supposed to decrease due to diabetes, increased significantly when plant extracts from the Apiaceae family were administered.

It was determined in the present study that there was a significant decrease in the GSH levels, as well as in CAT, SOD, GSH-Px and GSH-R activities in the pancreatic tissue, thus supporting the results of the studies [19, 20]. After the diabetic rats were treated with DT, a significant increase was observed in the antioxidant enzyme activities in pancreatic tissue. In terms of pancreatic antioxidant parameters, the 200 mg/kg/dose of DT was the most effective dose, and it was more effective than glibenclamide.

In the diabetes model formed by inducing STZ, damage and oxidative stress were observed in β-cells [1, 39] and the insulin immunoreactivity decreased [40]. A number of studies researching the effects of different plant extracts have reported that the damage caused by STZ is significantly observed and the insulin immunoreactivity increases [35, 40]. Similarly, in the present study, the morphology of the Langerhans islets was observed to deteriorate in the STZ-induced rats. In DT-treated diabetic

groups, the histopathological changes in the Langerhans islets and insulin immunoreactivity improved in parallel with the increase in dosage. It has been stated that the regeneration in the β-cells may be due to the decreasing of the oxidative stress by plant extract [40]. The effect of DT on the regeneration observed in Langerhans islets may be associated with in its antioxidant properties.

The use of herbal extracts in medicine are known to have antihyperglycemic effects on in vivo applications through various mechanisms including insulin secretion, an increase in insulin sensitivity, insulin biosynthesis, the regeneration in the islet cells, the use of the glucose in the skeletal muscle and the adipose tissue, and glucose absorption via the bowels [18]. The results of this study concluded that the most important antihyperglycemic action mechanism of the DT was associated with its healing power on beta cells within the pancreatic tissue langerhans islets. However, further studies assessing other possible mechanisms of action should be conducted. For this reason, there is a need for studies on these mechanisms.

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