Original Article

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Antidiabetic and Antioxidant Effect of the Aerial Parts of *Lysimachia verticillaris* and its Isolated Phenolic Compounds on Streptozotocin-induced Diabetic Rats

Lysimachia verticillaris'in Toprak Üstü Kısmının ve İzole Edilmiş Bileşiklerinin Streptozotosin ile İndüklenen Diyabetik Sıçanlarda Antidiyabetik ve Antioksidan Etkisi

Sıla Özlem ŞENER[1], Merve BADEM[2], Ufuk ÖZGEN[3], Nuriye KORKMAZ[2], Şeyda KANBOLAT[2], Gökçen KERİMOĞLU[4], Rezzan ALİYAZICIOĞLU[2], Engin YENİLMEZ[4], Esen SEZEN KARAOĞLAN[5]

[1]University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey
[2]Karadeniz Technical University Faculty of Pharmacy, Department of Biochemistry, Trabzon, Turkey
[3]Karadeniz Technical University Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, Turkey
[4]Karadeniz Technical University Faculty of Medicine, Department of Histology and Embryology, Trabzon, Turkey
[5]Atatürk University Faculty of Pharmacy, Department of Pharmaceutical Botany, Erzurum, Turkey

ABSTRACT

Objective: *Lysimachia* genus has been recorded to be used in diabetes, traditionally and to have antidiabetic effect and antioxidant effects. The aim of this study was to evaluate the antidiabetic and antioxidant effects of aqueous extract of *Lysimachia verticillaris* (LV) and its isolated compounds, the percentage of apoptosis and histological changes in pancreatic β-cells in rats with streptozotocin (STZ)-induced type 1 diabetes mellitus.

Methods: Male Spraque Dawley rats were divided into 6 groups. STZ (40 mg/kg) induced diabetic rats were treated orally with aqueous extract (400 mg/kg) and isolated compounds (20 mg/kg). To interpret antidiabetic effect, serum glucose and insulin levels were measured and morphological changes of pancreas were examined. Serum samples were analysed for catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) levels to investigate antioxidant effect. The terminal deoxynucleotidyl transferase (TDT)-mediated dUTP-biotin nick end-labeling (TUNEL) assays were performed to reveal apoptosis of pancreatic β-cells.

ÖZ

Amaç: *Lysimachia* cinsinin geleneksel olarak diyabet için kullanıldığı ve antidiyabetik ve antioksidan etkilere sahip olduğu kaydedilmiştir. Bu çalışmanın amacı, *Lysimachia verticillaris*'in (LV) su ekstresinin ve izole edilmiş bileşiklerinin streptozotosin (STZ) ile tip I diyabet oluşturulan sıçanlardaki antidiyabetik ve antioksidan etkilerini, pankreas β-hücrelerindeki apoptoz yüzdesini ve histolojik değişiklikleri değerlendirmektir.

Yöntemler: Erkek Spraque Dawley cinsi sıçanlar 6 gruba ayrılmıştır. STZ (40 mg/kg) ile indüklenen diyabetik sıçanlara, su ekstresi (400 mg/kg) ve izole bileşikler (20 mg/kg) oral yoldan uygulanmıştır. Deney sonunda antidiyabetik etkiyi yorumlamak için kan glukozu ve serum insülin düzeyleri tespit edilmiştir. Pankreastaki morfolojik değişiklikler incelenmiştir. Serum biyokimyasal parametreleri, katalaz (CAT), süperoksit dismutaz (SOD), glutatyon peroksidaz (GPX) açısından da antioksidan etkiyi araştırmak için analiz edilmiştir. Ayrıca pankreas β-hücrelerinin apoptozunun tespiti için terminal deoksinükleotidil transferaz aracılı dUTP-biyotin çentik uç etiketleme (TUNEL) deneyleri gerçekleştirilmiştir.

Address for Correspondence: Sıla Özlem ŞENER, University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey
E-mail: silashener@hotmail.com.tr **ORCID ID:** orcid.org/0000-0001-7679-7165

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Results: Oral administration of the extract and isolated compounds reduced high blood glucose levels (p<0.005). Morever, the treatments were resulted in increased serum insulin (p≤0.05), CAT (p≤0.05), SOD (p≤0.05) and GPX (p≤0.05). All applications with compounds, especially with quercetin 3-O-β-glucopyranoside reduced the morphological impairment of pancreas. The percentage of TUNEL positive cells was higher (p≤0.05) in the pancreatic islets of untreated diabetic group than others groups.

Conclusion: According to results, the extract and isolated compounds of LV displayed antidiabetic effect, while quercetin 3-O-β-glucopyranoside showed the highest antidiabetic effect. The antidiabetic effects of LV and phenolic compounds may be due to their antioxidant effects. Thus, LV and isolated compounds can be a potential source of herbal medicine for DM. However, it requires further investigations such as toxicological analysis studies and clinical trials.

Keywords: Antidiabetic, antioxidant, apoptosis, *Lysimachia verticillaris*, TUNEL

Bulgular: Ekstre ve izole bileşiklerin oral uygulamasının yüksek kan glikoz seviyelerini düşürdüğü saptanmıştır (p<0,005). Oral uygulamalar, serum insülin (p≤0,05), CAT (p≤0,05), SOD (p≤0,05) ve GPX (p≤0,05) düzeylerinin artışıyla sonuçlanmıştır. Ayrıca, kersetin 3-O-β-glikopiranozit için belirgin olmakla birlikte, uygulamaların pankreasın morfolojik bozukluğunu azalttığı saptanmıştır. TUNEL pozitif hücrelerin yüzdesi, tedavi edilmeyen diyabetik grubun pankreas adacıklarında diğer gruplara göre daha yüksek gözlenmiştir (p≤0,05).

Sonuç: Sonuçlara göre, LV'nin ekstresi ve izole edilmiş bileşikleri antidiyabetik etki gösterirken, kersetin 3-O-β-glikopiranozit en yüksek antidiyabetik etkiyi sergilemiştir. LV ve fenolik bileşiklerin antidiyabetik etkileri, DM'den kaynaklanan hasarlar üzerindeki antioksidan etkilerinden kaynaklanıyor olabilir. Bu nedenle, LV ve izole bileşikleri, diyabet ve komplikasyonları için potansiyel bir bitkisel ilaç kaynağı olabilir. Bununla birlikte, yeni bitkisel ilaçlar oluşturmak, toksikolojik analiz çalışmaları ve klinik araştırmalar gibi daha ileri araştırmalar gerektirir.

Anahtar Sözcükler: Antidiyabetik, antioksidan, apoptoz, *Lysimachia verticillaris*, TUNEL

Introduction

Diabetes mellitus (DM) which is a chronic and metabolic disease characterized by hyperglycemia gives rise to risk of cardiovascular, peripheral vascular and cerebrovascular disorders (1,2). Estimates indicated that 463 million people were living in the world with DM in 2019. The number is expected to increase to 700 million by 2045. DM is a global threat for the future due to health problems and economic burden it causes (3,4).

Oxidative stress in DM has been evidenced to occur due to increase of generation of reactive oxygen species and decrease of antioxidant defenses. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) are biomarkers to detect oxidative stress in DM (5). Deficient of insulin is directly related with the death of insulin-producing β-cells by apoptosis. The terminal deoxynucleotidyl transferase (TDT)-mediated dUTP-biotin nick end-labeling (TUNEL) tecnique is mostly used to detect apoptosis of pancreatic β-cells in DM (6,7).

Successful treatment of DM is not yet uncovered via synthetic drugs, therefore natural products have become more popular for treatment of DM. More than 400 medicinal plants have been proven to have hypoglycemic activity. Investigations of new antidiabetic natural herbal drugs are still remarkable owing to phytochemicals exhibiting potent and safe effects on DM (8).

The genus *Lysimachia* (Primulaceae) consists of 140-200 species worldwide (9) and is represented by 8 taxa in Turkish flora (10). *Lysimachia* species locally known as "karga otu, adi karga otu, altın kamış" in Turkey have been recorded to be used for expectorant, antipyretic, and wound healing purposes as well as against cough and bronchitis in Anatolian folk medicine (11). Also, *Lysimachia* genus has been used for the treatment of diabetes, hepatitis, urinary tract disorders, high blood pressure in the world, traditionally (12-15). *Lysimachia* genus has been reported to contain assorted secondary metabolites including flavonoids, triterpene saponins, steroidal saponins, benzodilactones, and quinones (14-17). Besides, several *Lysimachia* species have been demonstrated to exhibit many desirable biological activities such as anti-inflammatory, hepatoprotective, and vasorelaxant properties, etc. (18-20).

Previous studies showed that *Lysimachia* genus possessed therapeutic potential for the treatment of DM with traditional uses, antidiabetic and antioxidant effects. The methanolic extract of *Lysimachia candida* has proven to reduce insulin resistance in rats fed with high-fat high-fructose diet (21). It was reported that *Lysimachia foenum-graecum* and its isolated compound foenumoside B improved insulin sensitivity and metabolic profiles in ob/ob mice by PPARγ antagonism (22). *Lysimachia paridiformis stenophylla* has been evidenced to ameliorate lipid and carbohydrate metabolism in alloxan-induced diabetic mice due to its antioxidant and a-glucosidase inhibitory properties (23). It was demonstrated that the ethyl acetate fraction of *Lysimachia christinae* showed hypoglycemic effect based on its aldose reductase inhibitor effect (24). Previos study showed that *Lysimachia christinae* exhibited decreasing effect on lipid peroxidation levels and increasing effect on glutathione-*S* transferase (GST), GPX, SOD and CAT levels in alcohol-induced mice (25). *Lysimachia paridiformis* has been revealed to possess hepatoprotective properties in mice with CCl4-induced liver injury owing to its decreasing effect of glutamic-oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and increasing effect of SOD (26). Also, our previous study demonstrated that LV had high antioxidant capacity related with its phenolic compounds isolated (27).

Present study aims to investigate the antidiabetic effects of the aqueous extract of the aerial parts of LV and its phenolic compounds gallic acid, myrcetin 3-O-α-rhamnopyranoside and quercetin 3-O-β-glucopyranoside isolated in our previous

study (27), through detection of the biochemical parameters, the percentage of apoptosis and histological changes in pancreatic β-cells in rats with streptozotocin (STZ)-induced type 1 diabetes mellitus. To the best of our knowledge, it is the first study about antidiabetic properties of LV.

Methods

Drug and Chemicals

Streptozotocin was purchased from Sigma-Aldrich. Kits of insulin (Rat INS (Insulin) ELISA Kit: Catalog No: E-EL-R2466), CAT (Rat CAT (Catalase) ELISA Kit: Catalog No: E-EL-R2456), SOD (Rat SOD1 (Superoxide Dismutase 1, soluble) ELISA Kit: Catalog No: E-EL-R1424), GPX (Rat GPX1 (Glutathione Peroxidase) ELISA Kit: Catalog No: E-EL-R2491) obtanied from Elabscience. Other chemicals used were of analytical quality.

Plant Material and Extraction

The aerial parts of LV were collected from Kafkasör vicinity at altitude of 1.300 m (Artvin province, Turkey). The plant sample was identified by one of us (PhD. Ufuk Özgen). Voucher specimen (AEF 26311) is deposited at the Herbarium of Faculty of Pharmacy, Ankara University (Ankara, Turkey).

Air-dried and finely powdered aerial parts of the plant (100 g) were extracted with water (H_2O, 400 mL x8 h) at room temperature and the same process was repeated three times. The combined extracts were lyophilized to obtain approximately 15 g of the crude residue.

Experimental Animals

Fifty four male Sprague Dawley rats, weighing about 200-270 g obtained from Karadeniz Technical University, Surgical Researching Center. They were allowed to acclimatize for 14 days under standard house conditions of temperature (22±3 °C), humidity (40-65%) and 12- hour light and 12- hour dark cycle. The animals were fed with a standard pellet diet and administered water ad libitum. All experiments were executed in accordance with the ethical norms. The study was approval by the Institutional Ethical Committee of Karadeniz Technical University, Trabzon, Turkey.

Induction of Experimental Diabetes

Diabetes was induced by injecting streptozotocin (STZ) (40 mg/kg body weight) intraperitoneally. STZ was dissolved in freshly prepared 0.1 M sterile sodium citrate buffer (pH 4.5). Control rats were only injected with sodium citrate buffer. Two days after STZ administration, blood glucose level of each animal was measured by using automated glucose sensor machine (AccuCheck Active glucometer). Animals with blood glucose levels more than 200 mg/dL were perceived for the experiment (28).

Experimental Design

In the experiment, the rats were divided into 6 groups each consisting of 9 animals (Table 1).

Aqueus extract of LV and isolated compounds (gallic acid, myrcetin 3-O-α-rhamnopyranosid, quercetin 3-O-β-glucopyranoside) were dissolved in equal volume of saline and administered orally using an intragastric tube daily for 14 days.

At the end of the experiment, blood was collected and serum was separated for the estimation of levels of insulin and other biochemical parameters and pancreas was dissected out after euthanasia of rats by decapitation after anesthesia with ketamine.

Body Weight Analysis

The body weights of animals were measured in first and last days of the experiment.

Biochemical Analysis

The level of blood glucose concentration in mg/dL was evaluated by automated glucose sensor machine (AccuCheck Active glucometer) by collecting the blood from rat tail vein. For the estimation of insulin and other biochemical parameters, the blood was collected and serum was separated by centrifugation. Commercial diagnostic kits were utilized to determine of insulin, CAT, SOD, and GPX levels.

Histopathological Analysis

The pancreatic tissues obtained from all groups were fixed in formaldehyde 10% for 3 days and then dehydrated in 70%, 90%, 96%, 100% alcohol series. All the tissues applied xylene were embedded in paraffin and blocked. The paraffin-blocks were sectioned at thickness of 5 μm using microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany). The tissue sections were deparafinized with xylene and rehydrated in graded series of alcohol. Afterwards, all sections were stained with hematoxylin eosin (H & E). All histopathological analyzes were performed using a light microscope (Olympus BX-51; Olympus Co., Tokyo, Japan) equipped with an integrated camera (Olympus DP 71 Olympus Co., Japan). Injury in the pancreas was scored in terms of vacuole formation, pyknosis of nucleus and irregular pancreatic islet borders. Each criterion was rated from 0 to 3 (0, no damage, 1, mild damage, 2, moderate damage, 3, severe damage). Maximum score was evaluated as 9 (29).

Table 1. Control and diabetic animal groups

Groupings	Treatment
Group I	Normal rats received saline (0.5 mL/kg., p.o.)
Group II	STZ-induced diabetic rats received saline (0.5 mL/kg., p.o.)
Group III	STZ-induced diabetic rats received aqueus extract of LV (400 mg/kg., p.o.)
Group IV	STZ-induced diabetic rats received gallic acid (20 mg/kg., p.o.)
Group V	STZ-induced diabetic rats received myrcetin 3-O-α–rhamnopyranoside (40 mg/kg., p.o.)
Group VI	STZ-induced diabetic rats received quercetin 3-O-β-glucopyranoside (40 mg/kg., p.o.)

Apoptosis Analysis

Apoptosis was evaluated using the TUNEL technique. The technique was performed using In Situ Cell Death Detection Kit POD (catalog no: 11 684 817 910, Roche, Mannheim, Germany) and evaluated by light microscopy. Apoptosis was appraised for 300 islet cells in the four regions selected randomly under a magnification of X400 and values of apoptotis were expressed as percent (30).

Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation. Compatibility with normal distribution was determined using the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used to compare differences among the groups. Statistical significance level was considered as $p<0.05$.

Results

Results of Body Weight Analysis

At the end of the experiment, untreated diabetic group (II) showed significiant reduction of the body weight compared to control group (I) and the other groups had similar body weights compared to their initial weights although weight loss was observed (Figure 1).

Results of Biochemical Analysis

Oral administration of the extract and isolated compounds reduced high blood glucose levels ($p<0.005$) (Figure 2). Morever, the treatments resulted in increased serum insülin level compared to untreated diabetic groups ($p\leq0.05$) (Table 2). The highest hypoglycemic effect and healing effect were observed in quercetin 3-O-β-glucopyranoside treated group.

In comparison to the normal control, the untreated diabetic group displayed with considerable reduction of the levels of GPX ($p\leq0.05$), SOD ($p\leq0.05$) and CAT ($p\leq0.05$) (Table 2). The others groupsshowed increase in levels of antioxidant effective enyzmes, GPX ($p\leq0.05$), SOD ($p\leq0.05$) and CAT ($p\leq0.05$) (Table 2). The most significiant increase in the levels of CAT and SOD enyzmes was observed with quercetin 3-O-β-glucopyranoside, but highest level of GPX was detected with myrcetin 3-O-α-rhamnopyranoside.

Results of Histopathological Analysis

Microscopically, it was observed that control group (Group I) had regular langerhans islet margin consisting of normal appearing cells. In untreated diabetic rats (Group II), formation of cytoplasmic vacuoles and pyknosis of nucleus were observed, as well as irregular islets borders. Group III, IV and V were observed to have formation of vacuoles and pyknotic nucleus. Group III, IV, V and VIexhibited irregular langerhans islet margins more less than Group II. Group III, IV, V and VI had improved cell appearance of the islets. The histopathological appearance of most notable group (Group VI) was similar to the control group (Group I). The histological analysis of rat pancreas and scoring data are shown in Figure 3 and Table 3.

Results of Apoptosis Analysis

In the present study, TUNEL technique was used to evaluate apoptosis. The percentage of TUNEL-positive cells reduced in the pancreatic islets of groups compared with the untreated diabetic group (Figures 4, 5).

Discussion

Herbal medicines and their active ingredients have been proven to have antidiabetic effect owing to lots of mechanisms. So, natural products are getting more and more attractive for DM treatment day by day (31). Natural phenolic compounds prevent and treat DM through insulin-dependent or insulin-independent ways. Insulin-dependent effects of phenolic compounds on DM are expressed as activation of insulin signaling, stimulation of secretion of insulin, protection of pancreatic β-cell, reduction of β-cell apoptosis, supporting of β-cell proliferation, and decrease of oxidative stress. Insulin-independent effects of phenolic compounds for DM include inhibition of glucose absorption

Table 2. The values of biochemical parameters of control and diabetic groups

	Insulin (ng/mL)	CAT (pg/mL)	SOD (ng/mL)	GPX (pg/mL)
Group I	13.97±1.60$^{*\#}$	685.33±4.50$^{*\#}$	5.84±0.11$^{*\#}$	498.33 ±13.570$^{*\#}$
Group II	8.93±0.07$^{\$\#}$	428.01±10.39$^{\$\#}$	3.13±0.08$^{\$\#}$	187.33±8.08$^{\$\#}$
Group III	10.66±0.38$^{*\$}$	493.33±6.35$^{*\$}$	3.60±0.21$^{*\$}$	336.66±17.95$^{*\$}$
Group IV	10.86±0.12$^{*\$}$	537.00±9.53$^{*\$}$	5.15±0.14$^{*\$}$	444.01±36.59$^{*\$}$
Group V	11.20±1.02$^{*\$}$	586.01±18.08$^{*\$}$	5.74±0.22$^{*\$}$	531.00±15.52$^{*\$}$
Group VI	13.61±0.73$^{*\$}$	651.23±13.00$^{*\$}$	6.44±0.06$^{*\$}$	528.21±17.32$^{*\$}$

CAT: Catalase, SOD: Superoxide dismutase, GPX: Glutathione peroxidase

Table 3. The values of biochemical parameters of the control and diabetic groups

	Group I	Group II	Group III	Group IV	Group V	Group VI
Injury in pancreas	0.00±0.00	7.20±0.42*	4.31±0.31*	3.09±0.21*	3.30±0.28*	0.62±0.17*

(values are mean ± SD, n=9), *P≤0.05 (compared to diabetic group)

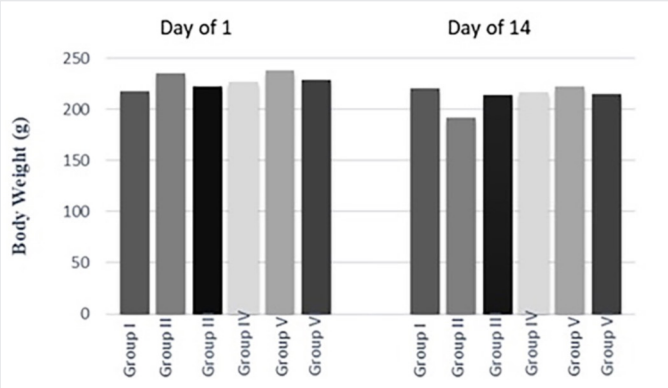

Figure 1. Column graph represents the body weight of the control and diabetic groups

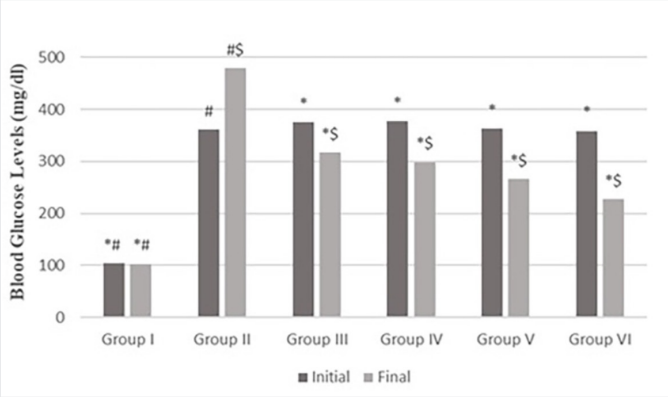

Figure 2. Column graph represents the blood glucose levels of the control and diabetic groups. *P≤0.05 (compared to control group), $P≤0.05 (compared to diabetic group), #P≤0.05 (compared to control group with diabetic group)

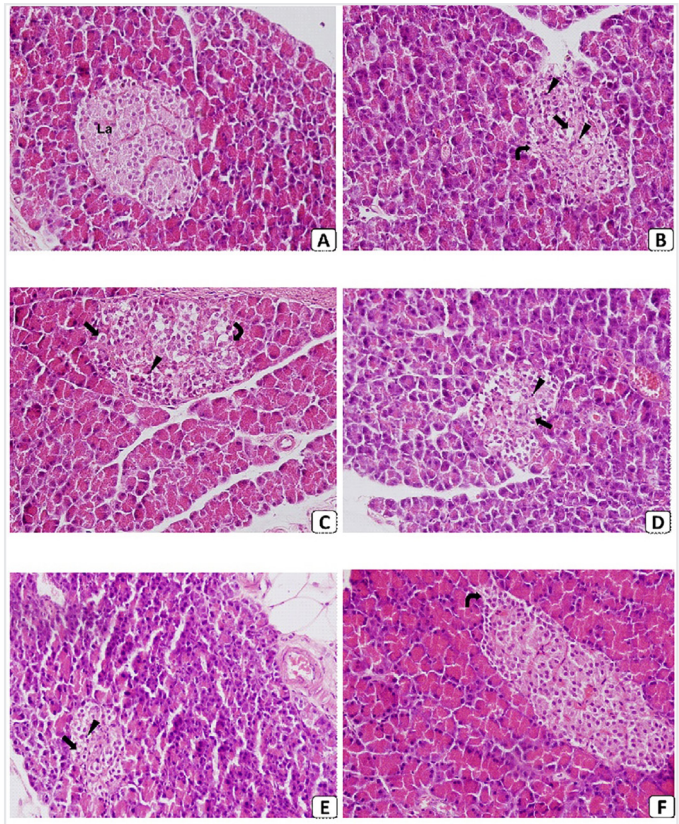

Figure 3. Group I (A), Group II (B), Group III (C), Group IV (D), Group V (E), Group VI (F) Langerhans islet (La), vacuole (arrow), pyknosis of nucleus (arrow head), irregular islet borders (curved arrow) (H & E x400). H & E: Hematoxylin/Eosin

or digestive enzymes, adjustment of intestinal microbiota, and replacement of inflammation response (32).

Gallic acid has been uncovered to have ameliorating effect on hyperglycemia in diabetic rats with STZ-induced pancreatic dysfunction (33). An *in vivo* study of STZ-induced type I diabetes in rats reported that gallic acid exhibited healing effects leading immuno- and thrombo-regulatory responses in DM through the modulation of purinergic signaling pathways (34). Gallic acid, one of the natural phenolic compounds, was used for positive control for the study due to previous literature.

It was reported that administration of myricetin gave rise to reduction of hyperglycemia in STZ-induced diabetic rats. Myricetin has been proven to alleviate high glucose levels and remedy insulin resistance by modulation of β-endorphin generation in fructose-induced insulin-resistant rats (34). Myricetin has been evidenced to protect from tert-butyl hydroperoxide-induced oxidative stress in erythrocytes of patients with type 2 DM. Myricetin has been revealed to have inhibitory effect on aldose reductase and α-amylase (35).

The antidiabetic effect of quercetin arise from the stimulation of glucose uptake related with MAPK insulin-dependent mechanism (36). Quercetin has been found to ameliorate oxidative stress in STZ-induced diaberic rats (37). A further study demonstrated that quercetin displayed beneficial effect in STZ-induced diabetic nephropathy via antioxidative mechanism (38). It was reported that quercetin-3-O-glucoside cured postprandial glycemic control in rats and decreased glucose uptake in Caco-2 cells owing to reduce the expression of glucose transporters (39).

The STZ, a naturally occurring compound, is used to create diabetes in experimental animals. STZ possesses selective cytotoxicity to pancreatic β-cells which adjust blood glucose levels via insulin hormone (40). STZ transported to pancreatic cells by glucose transporter 2 brings about activation of poly adenosine diphosphate ribosylation and nitric oxide release. The STZ causes devastation of pancreatic cells by necrosis and ultimately creates insulin-dependent diabetes (41).

The DM characterized by rising levels of glucose in blood arises from inadequate production and action of insulin (42). STZ induces significant reduction of plasma insulin levels in rats (43). The present study showed that oral administration of the extract and isolated compounds exhibited significantly decreasing effect on blood glucose levels and healing effects of insulin levels. As mentioned above, myrcetin and quercetin have been proven to

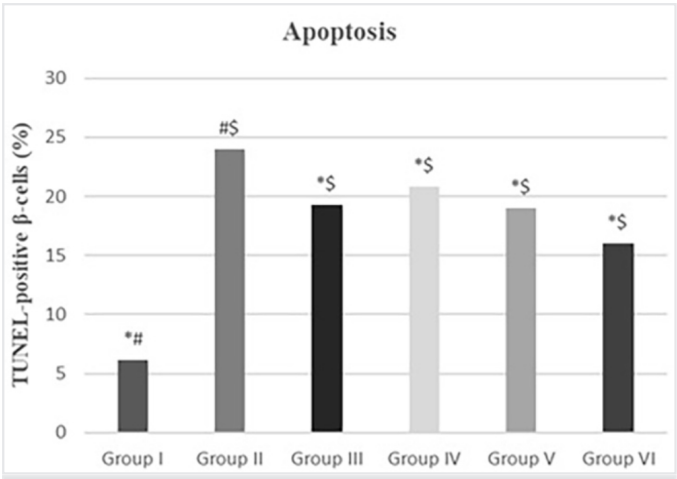

Figure 4. Column graph represents the percentage of TUNEL-positive β-cells in the control and diabetic groups. *P≤0.05 (compared to control group), $P≤0.05 (compared to diabetic group), #P≤0.05 (compared to control group with diabetic group)

possess antidiabetic effect via many mechanisms such as MAPK insulin-dependent mechanism, modulation of β-endorphin generation, antioxidant effect and enyzme inhibitions (35-39). The antidiabetic effect of the extract on blood glucose levels may be related to myricetin and quercetin derivative compounds in its content.

The STZ-induced diabetes gives rise to loss of body weight. The significative loss of body weight may be related with increment of muscle wasting and reduction of tissue proteins (44). In present study, untreated diabetic group (II) showed significiant reduction of the body weight compared to control group (I). Significant reduction of body weight may be related to the effects of STZ such as muscle wasting and reduction of tissue proteins. However, the others groups owned body weight similar to initial weight although they lost some weight. This situation indicated that the extract and its isolated compounds improved some toxicological effects of STZ.

In diabetes, oxidative stress is increased by diverse mitochondrial, enyzmatic and non-enyzmatic sources (45,46). The potential reason of oxidative stress includes increase of free radicals and impairment of antioxidant defense mechanism due to reduction in the levels of GPX, SOD and CAT (47,48). GPX metabolizes peroxide to water and oxgen. Alterations of the enyzme in DM induce the cells to prone for oxidative stress and cell injury (49,50). SOD, a primary defender against superoxide on cell injury, is responsible for catalyzing superoxide anion into hydrogen peroxide and molecular oxygen (51). CAT catalyses H_2O_2 into water and oxygen. Deficieny of CAT causes β-cell dysfunction and finally diabetes (42). While the levels of antioxidant effective enzymes (GPX, SOD, CAT) were decreased in untreated diabetic group, the levels of antioxidant effective enzymes (GPX, SOD, CAT) were increased in the other treatment groups in the present study. The antioxidant effects of myrcetin and quercetin were proven (35-39). The antidiabetic effect of myrcetin 3-O-α-

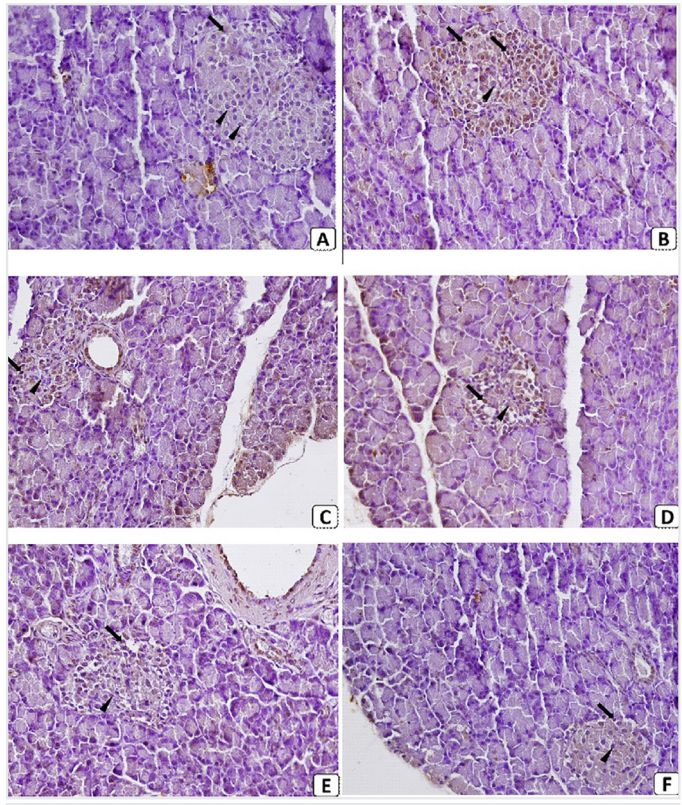

Figure 5. Group I (A), Group II (B), Group III (C), Group IV (D), Group V (E), Group VI (F) Apoptotic cells (arrow), normal cells (arrow head) (TUNEL, x400)

rhamnopyranoside and quercetin 3-O-β-glucopyranoside groups are probably directly related to the antioxidant effect of these phenolic compounds. Similarly, these phenolic compounds in the extract contributed to the antidiabetic and antioxidant effects of the extract.

Values are determined from day 14 after STZ administration, (values are mean ± SD, n=9), *P≤0.05 (compared to control group), $P≤0.05 (compared to diabetic group), #P≤0.05 (compared to control group with diabetic group)

Pancreatic β cell damage by apoptosis is evaluated as a most important factor for improving of hyperglycemia and diabetes (52). The exract and all isolated compounds were proven to have healing effect on STZ-induced pancreatic β cell damage with this study.

Previous studies reported microscopic analyses that Lymphocyte filteration, nucleus pyknosis, cytoplasmic vacuolization and irregular margins were observed in the islet beta cells of untreated diabetic rats (53). Present study revealed that Group III, IV, V and VI showed ameliorating effect when compared to negative control group, histopathologically. Group IV, V and Group VI displayed healing effects on langerhans islet margins. Most notable group, Group VI, decreased pathological effects related with diabetes considerably and exhibited improving effect on langerhans islet margins and cell appearance in most of the islets, similar to the control group (Group I). Thus, histopathological

and apoptosis analyzes supported the antidiabetic effect of LV and its isolated compounds.

Consequently, LV and its active ingredients, gallic acid, myrcetin 3-O-α-rhamnopyranoside and quercetin 3-O-β-glucopyranoside, possess therapeutic potential in DM in accordance with previous literature about importance of phenolic compounds and therapeutic potential of *Lysimachia* genus.

Conclusion

Successful treatment of DM is not yet found and the prevalence of DM is increasing day by day, thus DM is still a global problem. Accordance with traditional uses, the extract and isolated compounds of LV display antidiabetic effect through antioxidant properties and ameliorating effect on STZ-induced histopatholigical changes and pancreatic β cell damage. Thus, LV and isolated compounds especially quercetin 3-O-β-glucopyranoside can be a potential source of herbal medicine for the global problem and related complications through antioxidative mechanism or diminution of apoptosis. Further investigations and human trials are required for therapeutic effect of LV and isolated compounds on the global problem.

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Ethics

Ethics Committee Approval: All experiments were executed in accordance with the ethical norms. The study was approval by the Institutional Ethical Committee of Karadeniz Technical University, Trabzon, Turkey.

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Authorship Contributions

Surgical and Medical Practices: S.Ö.Ş., M.B., U.Ö., R.A., G.K., E.Y., Concept: S.Ö.Ş., Design: S.Ö.Ş., M.B., N.K., Ş.K., Data Collection or Processing: S.Ö.Ş., M.B., U.Ö., N.K., Ş.K., R.A., Analysis or Interpretation: S.Ö.Ş., M.B., N.K., Ş.K., R.A., G.K., E.Y., Literature Search: S.Ö.Ş., U.Ö., E.S.K., Writing: S.Ö.Ş., E.S.K.

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