Van Vet J, 2019, 30 (2) 121-125

Van Veterinary Journal

http://dergipark.gov.tr/vanvetj

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Investigation of the Antidiabetic Effects of Mistletoe (*Viscum album L.)* Extract in Experimental Diabetes in Rats

Avin Kawa AHMED* Handan MERT Nihat MERT

Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Biochemistry, Van, Turkey

Received: 14.06.2019 Accepted: 19.07.2019

ABSTRACT Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies distinguished by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. Extracts of the *V. album* leaves was reported to exert a beneficial effect to alleviate the symptoms of diabetes in local medicines. The aim of present investigation is to evaluate the antidiabetic and antioxidant activities of *V. album* extracts on streptozotocin (STZ) induced diabetic rats. Thirty two female Wistar albino rats, aging 6-7 weeks, were included in the present study. The rats were randomly divided into 4 groups: Group I (Control), Group II (*V. album*), Group III (Diabeteic), Group IV (Diabetic + *V. album*). After experimental period, all animals were decapitated, blood was collected and sera were separated. Serum concentrations of total cholesterol, high density lipoprotein, cholesterol, triglyceride, folic acid, vitamin D, vitamin B_{12} and glucose were determined by using automatic analyzer. Total Antioxidant Capacity (TAC) and Total Oxidative Status (TOS) analyses were measured ELISA. The levels of glucose ($p \leq 0.05$), vitamin B_{12} ($p \leq 0.05$), TOS ($p \leq 0.05$) were significantly decreased and TAC ($p \leq 0.05$) increased in diabetic+V. album group compared to diabetic group. In conclusion extracts of *V. album* have antidiabetic effects that significantly decreased glucose level when treated with *V. album* only 20 days of experiment. *Viscum album* had a stronge antioxidant activity that can help to prevent the formation of diabetic complications.

Keywords: *Diabetes, Lipoproteins, Total antioxidant capacity, Total oxidative status, Viscum album, Vitamins*

ÖZ

Ratlarda Deneysel Diyabette Ökseotu (*Viscum album L.*) Ekstresinin Antidiyabetik Etkilerinin Araştırılması

Diyabet çoklu etiyolojisi, karbonhidrat, yağ ve protein metabolizması bozuklukları ve kronik hiperglisemi ile seyreden metabolik bir hastalıktır. *V. album* (ökse otu) yaprak ekstreleri, diyabet semptomlarını hafifletmek, diyabete yararlı bir etki sağlamak için kullanılan yöresel bir bitkidir. Bu çalışmanın amacı, *V. album* ekstraktlarının streptozotosin (STZ) kaynaklı diyabetik sıçanlar üzerindeki antidiyabetik ve antioksidan aktivitelerini araştırmaktır. Araştırmada 32 adet, dişi 6-7 haftalık Wistar sıçanları kullanıldı. Sıçanlar rastgele 4 gruba, ayrıldı. Grup I (Kontrol), Grup II (*V. album*), Grup III (Diyabetik), Grup IV (Diyabetik + *V. album*). Deneme sonunda tüm gruplardaki sıçanların kan örnekleri alınıp hemen serumları ayrıldı. Total kolesterol, HDL, trigliserit, folik asit, vitamin D, vitamin B_{12} ve glukoz düzeyleri otoanalizörde saptandı. TAC ve TOS analizleri ELISA'da ölçüldü. Diyabetik+*V. album* grubunda diyabet grubuna göre glukoz ($p \leq 0.05$), vitamin B_{12} ($p \leq 0.05$) ve TOS ($p \leq 0.05$) düzeyleri önemle azalırken, TAC düzeyi önemle yükseldi ($p \leq 0.05$). Sonuç olarak; *V. album* ekstreleri antidiyabetik etkilere sahiptir. Sadece 20 günlük deneme süresince *V. album* ile tedavi edildiğinde glukoz seviyesini önemli ölçüde azaltmıştır. *Viscum album*, diyabetik komplikasyonların oluşumunu önlemeye yardımcı olabilecek güçlü bir antioksidan aktiviteye sahiptir.

Anahtar Kelimeler: *Diyabet, Lipoproteinler, Ökse otu, Toplam antioksidan kapasite, Toplam oksidatif durum, Vitaminler*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiology distinguished by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO 2014). By 2035, the number of diabetes cases worldwide is expected to increase to 592 million. In addition to risk of death from hyperglycaemic crisis, chronic hyperglycemia from diabetes can lead to many long term health issues (Aschner et al. 2014). These sequelae include: Cardiovascular disease, and an increased risk of heart attack or stroke; chronic kidney disease leading to end-stage renal disease; urinary problems;

Corresponding author: Handan MERT
Van Yüzüncü Yıl Üniversitesi, Veteriner Fakültesi, Biyokimya AD, Van, Turkey. e-mail: hmert@yyu.edu.tr
**This research summarized from first author's master thesis.*

peripheral neuropathy; skin infections, especially foot ulcers, that are difficult to heal, with the eventual need for lower-extremity amputation; retinopathy leading to vision loss; and sexual dysfunction (Widatalla et al. 2009; Kollias and Ulbig 2010).

The currently available antidiabetic agents include sulfonylureas, biguanides, thiazolidinediones and alpha glucosidase inhibitors and are widely used to control the hyperglycemia. Traditional medicinal plants having antidiabetic properties can be a useful source for the development of safer and effective oral hypoglycemic agents. More than 350 traditional plants are used in the treatment of diabetes mellitus, which have been recorded (Jung et al. 2006). Only a small number of these have received scientific and medical evaluation to assess their efficacy. However, plant remedies are the mainstream of treatment in underdeveloped regions. One of the great advantages of medicinal plants is that these are readily available and have very low side effects. The phytoconstituents showing hypoglycemic effect includes glycosides, alkaloids, terpenoids, flavonoids, carotenoids, that are frequently implicated as having antidiabetic effect (Malviya et al., 2010).

Viscum album belongs to the family Loranthaceae. It is also called as mistletoe. *Viscum album L.* is an evergreen dioecious small shrub growing half parasitically on a tree host in temperate Europe and Western Asia. In Anatolia of Turkey, the plant grows on different host trees and shrubs (Miller 1982). *Viscum album* is locally known as "ökse otu" and widely utilized for the treatment of epilepsy, diabetes mellitus, cancer, hypertension, headache, and rheumatoid arthritis in various regions of Turkey (Baytop 1984; Simsek et al. 2004). Extracts of the *V. album* leaves was reported to exert a beneficial effect to alleviate the symptoms of diabetes in local medicines (Gray and Flatt 1999; Orhan et al. 2005). *Viscum album* has been shown also to relieve the diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice (Swanston-Flatt et al. 1989).

The aim of present investigation is to evaluate the antidiabetic and antioxidant activities of *V. album* extracts on streptozotocin (STZ) induced diabetic rats.

MATERIALS and METHODS

Plant materials

Viscum album extract was purchased from VSM (Geneesmiddelen BV, Holland, *Viscum album* V30) herbal drug preserved at room temperature protected from light until use experiments.

Experimental animals

Thirty two female Wistar rats, aging 6-7 weeks and weighing 200-250 g, were included in the present study. The animals were provided by the Experimental Animal Center of Yuzuncu Yil University, Turkey. All the animals were maintained under laboratory conditions of temperature (22±2◦C), humidity (45±5 %) and 12h day: 12h night cycle and were allowed access to food as standard pellet diet and water were available ad libitum throughout the 20 days of experimental period.

Experimental design and treatment schedule

Rats were made diabetic by a single intraperitoneal injection of 45 mg/kg STZ (Sigma, USA), pH: 4.5 in 0.1M citrate buffer in a volume of 1ml/kg bw (Karabay et al. 2006). After 72 hours the blood was taken by tail puncture and the plasma glucose level of each rat was determined by blood glucose measurement using a blood glucose monitoring system (eBsensor eB-G). Rats with a plasma glucose range of 250 mg/dl were considered diabetic and included in the study.

The rats were randomly divided into 4 groups:

Group I (control, n = 8) was fed with normal pellet diet,

Group II (*V. album*, n= 8) rats treated with *V. album* 10 mg/kg (Oztabak 2005) were given orally for 20 days of experimental period,

Group III (diabetic, n = 8), rats were injected with single dose of STZ 45 mg/kg, intraperitoneally,

Group IV (Diabetic + *V. album*, n= 8), rats were injected with single dose of STZ 45mg/kg, intraperitoneally, and rats treated with *V. album* 10 mg/kg were given orally for 20 days of experimental period.

After 20 days of administration, all animals were decapitated, blood was collected and serum was separated immediately.

Biochemical analysis

At the end of experimental period, rats were fasted overnight, anaesthetized, with an intraperitoneal injection of ketamine hydrochloride (70 mg/kg b.w /rat) with 8 mg/kg dose of xylazine. The animals were continually monitored until total loss of consciousness was reached, as indicated by a total lack of response after a foot pinch then blood samples taken through the heart puncture of rats. Blood was collected, allowed to clot on ice and subsequently subjected to centrifugation (Allegra X-15R Centrifuge 4000 RPM at 4◦C for 10 min) serum was collected and stored at −80 °C until assayed. Serum concentrations of total cholesterol, high density lipoprotein cholesterol, triglyceride, folic acid, vitamin D, vitamin B12 and glucose were determined by using automatic biochemical analyzer (Abbott Architectci 16200, Spectrophotometer, Germany). TAC and TOS were determined using a commercial kit (Rel Assay Diagnostic, Van, Turkey).

The percentage ratio of TOS to TAC indicated oxidative stress index (OSI), an indicator of the degree of oxidative stress (Erel, 2004; 2005). To perform the calculation, the resulting unit of TAC (mmol Trolox equivalent/litre) was converted to μmol equivalent/litre and the OSI value was calculated using the following formula:

OSI = [(TOS, μmol/L)/(TAC, mmol Trolox equivalent/L) x100] (Erel, 2005).

Statistical analysis

The data reported were submitted to ANOVA using Statistica-Statsoft® 7.0 and represent the means ± standard deviation values. Differences among treatments were analyzed by Tukey's test. A P value of 0.05 was used as the cut-off for statistical significance.

RESULTS

The levels of glucose ($p \leq 0.05$), vitamin B12 ($p \leq 0.05$), TOS ($p \leq 0.05$) were significantly decreased and TAC ($p \leq 0.05$) increased in diabetic+*V. album* group compared to diabetic group. Vitamin D, cholesterol, HDL levels were not significant among the groups. Folic acid level was significantly higher in diabet and diabetes + *V. album* group compared to the other two groups. The triglyceride level of *V. album* group was significantly higher than the other three groups (Table 1).

Table 1. Changes in the serum glucose, folic acid, vitamin B12, vitamin D, total cholesterol HDL and triglyceride, TOS, TAC levels in different groups

Parameters	Control Mean ± SD (n=8)	*V. album* Mean ± SD (n =8)	Diabetic Mean ± SD (n=8)	Diabetic+*V. album* Mean ± SD (n=8)
Glucose (mg/dl)	193.14± 31.23a	231.71± 61.41a	700.50± 164.08c	514.62±235.15b
Folic Acid (ng/ml)	15.01± 0.85a	13.84± 0.59a	16.32± 1.16b	16.28± 1.64b
Vitamin B_{12} (ng/ml)	355.85± 24.92[a]	355.42± 32.23[a]	543.62± 109.44[b]	410.75± 109.54[a]
Vitamin D (ng/ml)	71.11± 6.79[a]	65.34± 10.02[a]	72.20± 9.51[a]	68.57± 14.45[a]
Cholesterol (mg/dl)	66.85± 7.60[a]	65.28± 8.17[a]	59.37± 12.63[a]	63.25± 8.82[a]
Triglyceride (mg/dl)	92.85± 27.55[a]	122.0± 51.73[b]	65.12± 25.69[a]	98.12± 20.82[a]
HDL (mg/dl)	43.40± 5.88a	42.20± 5.58a	37.0± 9.24a	39.77± 5.35a
TOS (µmol H2O2 Eq/L)	2.91±0.25c	3.21±0.18c	4.15±0.37a	3.91±0.22b
TAC (mmol Trolox Eq/L)	1.44±0.11[b]	2.15±0.16[a]	0.98±0.14[c]	1.35±0.17[b]
OSI	0.202±0.061[b]	0.149±0.094[c]	0.423±0.079[a]	0.290±0.088[b]

a, b, c: The difference between group averages with different letters in the same row is statistically significant (p≤0.05)

DISCUSSION

Diabetes mellitus is a serious chronic metabolic disease, in 2014, about 387 million people worldwide, or 8,3% of the adult population aged 20-79 years, are estimated to have diabetes with increasing incidence (IDF 2015). DM is featured with high blood glucose levels that result from insulin resistance in peripheral tissues or impaired insulin synthesis in pancreas (De Silva et al. 2012). In addition to the impairment in glucose and carbohydrate metabolism, the disease causes abnormalities in the metabolism of lipid and protein. The high blood glucose is also associated with generation of reactive oxygen species and consequent oxidative damages particularly in liver, kidney, and pancreas (Kakkar et al. 1998).

STZ an antibiotic produced by Streptomyces achromogenes, possesses pancreatic β-cell cytotoxic effect (Weiss 1982). STZ has been widely used for inducing diabetes mellitus in a variety of animals. STZ causes degeneration and necrosis of pancreatic β-cells (Uchigata et al. 1982). In this study it was clearly demonstrated that STZ at a dose of 45 mg/kg was able to provoke a sustained hyperglycemia in both diabetic group and diabetic +*V. album* group, the level of serum glucose was increased significantly ($p<0.05$) in diabetic group and diabetic+*V. album* group in comparison with control group (Table 1). Diabetic rats have high blood glucose as 700,50 mg/dl and it was significantly decreased glucose level when treated with *V. album* to 514,64 mg/dl in only 20 days of experiment, this decline was statistically significant ($p \leq 0.05$). In previous studies had reported a significant decrease of plasma glucose was not clearly demonstrable in this insulin-deficient model (Swanston-Flatt et al. 1989), which indicates that leaf and stem of mistletoe contain water soluble natural product(s) which directly stimulate insulin secretion from clonal β-cells. Mistletoe leaves was reported to exert a beneficial effect to alleviate the symptoms of diabetes in local medicines worldwide (Gray and Flatt 1999). *Viscum album* is a traditional plant using for the treatment of diabetes. 1-10 mg/ml aqueous extracts can stimulate the insulin secretion. The presence of insulin-releasing natural compounds may contribute the antidiabetic effects of this plant. *Viscum album* relieves the diabetic symptoms the polyphagia, body weight loss and polydipsia. *Viscum album* extracts act in the early stage of the insulin secretory pathways Gray and Flatt (1999) had found water soluble heat resistant insulin releasing compounds in *V. album* and suggested this plant as a new oral hypoglycemic agent. *V. album* extracts have antidiabetic effects.

One of the common mechanisms suggested in the pathogenesis of diabetes diseases is the formation of reactive oxygen species (ROS) that leads to oxidative stress (Tiwari et al. 2013). In the recent years there has been an increased interest in ROS generation and its role in the development of complications of diabetes. In relation to this concern, Giacco and Brownlee (Giacco and Brownlee 2010) showed that persistent hyperglycemia can enhance the oxidative stress by increasing glucose auto-oxidation, non-enzymatic protein glycation, and activation of polyol pathway. Numerous studies have demonstrated that oxidative stress plays an important role in the pathogenesis and progression of diabetes and its complications (Simmons 2006; Kaneto et al. 2007). Oxidative stress has recently been shown to be responsible, at least in part, for the β-cell dysfunction caused by glucose toxicity. Under hyperglycemia, production of various reducing sugars such as glucose-6-phosphate and fructose increases through glycolysis and the polyol pathway. During this process, ROS are produced and cause tissue damage (Hunt et al. 1990). However, besides the increased production of reactive species following hyperglycaemia, the intracellular antioxidant defense is of great importance in disease progression and development of diabetes complications (Gupta and Chari 2005). In the presented study the effect of *V. album* on the antioxidant status of the STZ-diabetic rats was investigated. For this purpose the effects of *V. album* extract on activity of TAC and TOS of diabetic rat's serum

were studied. TOS and TAC are biomarkers of oxidative stress that are measured easily, fast and economic. In this sense, TOS is the measurement of different oxidant species in an organism (Aslan et al. 2014; Barbosa et al. 2014) that was shown to be of help in the diagnosis of different pathologies (Erel 2005). TAC determinations are of help to study the capacity of known and unknown antioxidants and their additive, synergistic and/or antagonistic actions, in chemical and biological systems (Fraga et al. 2014). Currently TAC assays have applications in human and veterinary medicine to evaluate overall defense status against oxidative stress and biomedical sciences (Bartosz and Bartosz 1998).

Diabetes has so many complications as diabetic retinopathy, nephropathy, neuropathy and hypertension. There is a correlation between complications and oxidant/antioxidant status of the body. The TOS ratio increased in diabetic patients and especially patients with retinopathy. Unlike the amount of TAC decreased in DM. This negative situation have important role on the formation of DM and its complications. Logically TAC levels are reduced while the TOS levels are increased in DM. In this study similar results were determined the TAC levels which decreased in diabetic rats, but quantity of TAC increased in diabetes when *V. album* was given. TAC is a measure of the antioxidant capacity of biological samples analyzed, it is not a single compound. But it would be better to compare the levels of individual antioxidant with TAC. In this study, the levels of TOS were significantly increased ($p \leq 0.05$) and TAC (≤ 0.05) decreased in diabetic group compared to diabetic+*V. album* group. *V. album* extracts samples revealed significant antioxidant and radical-scavenging properties and reported oxidative stress is associated with the development and progression of diabetes mellitus.

Oxidative stress index (OSI) was defined as the ratio of TOS level to TAC level. In this study the highest ratio was found in diabetic group, because of producing so much oxidant in there disease. But when rats consumed *V. album* during diabetes these ratio decreased, it is meant in that *V. album* had antioxidant compound, effects and had positively changed this ratio. This is very impressive results, because *V. album* had positive effects to diminish the amount of oxidant produced in DM, which all of them are so harmful and cause the diabetic complications. In addition to the impairment in glucose and carbohydrate metabolism, the disease causes abnormalities in the metabolism of lipid and protein (Kuzuya et al. 2002). In Japan totally 1771 men and women were analyzed for lipid profile detection, diabetic men total cholesterol and HDL in women triglyceride were the most predictive parameters, that lipid component of serum, lipid metabolism have significant changes during DM. In the presented study cholesterol, triglyceride and HDL levels were not significantly affected by *V. album* or DM (Sone et al. 2012).

Vitamin B_{12} or cobalamin plays very important role in DNA synthesis neurological function and optimal haemotopoiesis. Deficiency of this vitamin will result in disruption of methylation process and accumulation of homocysteine. Thus the vascular endothelium will be toxically affected. There is a correlation between vitamin B_{12} deficiency and type 1 and 2 DM (Liu et al. 2006). Bhatt and Linnell (Bhatt and Linnell 1983) and Bhatt et al. (Bhatt et al. 1983) also stated that plasma vitamin B_{12} levels were almost doubled in the diabetic rat and 'abnormal' vitamin B_{12} metabolism was suggested as one explanation for the reported elevated methylmalonic acid levels in the same animals. The vitamin B_{12} status of the diabetic animals in other study did not differ from that of control animals (Bailey et al. 1989). In the presented study within 20 days, vitamin B_{12} levels were not changed in all group except diabetes group, diabetes group had the highest amount of vitamin B_{12} levels.

Some researchers said that folic acid supplementation of diabetic people were significantly reduced homocysteine levels and lowered cardiovascular risks (Audelin and Genest 2001). Folic acid diminishes the risk of neural tube defects of the fetus. Supplementing rats with folic acid decreased teratogenicity induced by diabetes mellitus. DM affects folate metabolism, create a functional folic acid deficiency in embryo. In the presented study the folic acid levels were not changed drastically, only slight alterations were found (Table 1).

Vitamin D is an important vitamin for the normal growth and skeletal system. But in the last years the function and magnificent effect to almost all systems were described. Vitamin D is known as sunshine vitamin and has great vital function on the regulation and absorption of calcium and phosphorous, facilitate the normal role of the immune system is very surprising, too. Reduction the risk of muscle sclerosis, decreasing heart disease, and the regulating mood and warding off depression and fibromyalgia and significant effect on pancreatic β-cell function are the sum of function of vitamin D. Some studies showed that adequate vitamin D levels in the blood have positive effect on the pancreatic β-cell. The person has enough vitamin D they start normal pancreatic function. If one has normal blood vitamin D levels, pancreas functions properly. When vitamin D receptors were removed, inadequate production of insulin was measured (Zeitz et al. 2003). Vitamin D influences the regulation of calcium in the blood and Ca^{+2} help control insulin secretion So if one has altered Ca^{+2} levels, β-cell function will be spoiled, when the blood level of Ca^{+2} changes, the β-cell function will be affected. If someone has ≥25 ng/ml blood vitamin D level can have low detection rate in type 2 diabetes (Zeitz et al. 2003; Davidson et al. 2013). In this study, there was no importance in terms of vitamin D levels among the groups.

CONCLUSION

In the presented study, diabetic rats have high blood glucose and it was significantly decreased glucose level when treated with *V. album* in only 20 days of experiment. Additionally *V. album* extracts samples revealed significant antioxidant and radical-scavenging properties and reported oxidative stress is associated with the development and progression of diabetes mellitus. Furthermore, the OSI clearly showed that *V. album* had a stronge antioxidant activity that can help to prevent the formation of diabetic complications.

ACKNOWLEDGEMENT

This research was supported by the Van YYU Directory of Scientific Research Project as (2016- TYL-5198) Project number. The same study was presented at IMCOFE IV, Roma, ITALYA, 23-25 Ağustos 2017.

REFERENCES

Aschner P, Aguilar-Salinas C, Aguirre L, Franco L, Gagliardino JJ, de Lapertosa SG, Seclen, S, Vinocour M (2014). Diabetes in South and Central America, an update. *Diabetes Res Clin Pract,* 103 (2), 238-243.

Aslan R, Kutlu R, Civi S, Tasyurek E (2014). The correlation of the total antioxidant status (TAS), total oxidant status (TOS) and paraoxonase activity (PON1) with smoking. *Clinical Biochemistry*, 47(6), 393-397.

Audelin MC, Genest J (2001). Homocysteine and cardiovascular disease in diabetes mellitus. *Atherosclerosis*, 159 (2), 497-511.

Bailey LB, MolLoy A, Scott J, Rice D (1989). Streptozotocin-induced diabetes is not a model for methylmalonic acidaemia. *J Inherit Metab Dis*, 12(4), 429-435.

Bhatt HR, Linnell JC (1983). Induction of ethylmalonic acidaemia with streptozotocin: a possible experimental model for the study of methylmalonic acidaemia. *J Inherit Metab Dis*, 6, Suppl, 2.

Bhatt HR, Linnell, JC, Matthews DM (1983). Can faulty vitamin B12 (Cobalamin) metabolism produce diabetic neuropathy? *The Lancet*, 3, 572.

Barbosa CF, Tonin AA, Da Silva AS, De Azevedo MI, Monteiro DU, Waczuk EP, Faccio L (2014). Diphenyl diselenide and sodium selenite associated with chemotherapy in experimental toxoplasmosis: influence on oxidant/antioxidant biomarkers and cytokine modulation. *Parasitology*, 141 (13) 1761-1768.

Bartosz G, Bartosz M (1998). Antioxidant activity: What do we measure? *Acta Biochim Pol*, 46 (1), 23-29.

Baytop T (1984). Therapy with Medicinal Plants in Turkey (Past and Present), *Publications of IX Istanbul University,* 243.

Davidson MB, Duran P, Lee ML, Friedman TC (2013). High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis. *Diabetes Care*, 36 (2), 260-266.

De Silva DD, Rapior S, Hyde KD, Bahkali AH (2012). Medicinal mushrooms in prevention and control of diabetes mellitus. *Fungal Diversity*, 56 (1), 1-29.

Erel O (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, 38, 1103-1111.

Fraga CG, Oteiza PI, Galleano M (2014). In vitro measurements and interpretation of total antioxidant capacity. *Biochim Biophys Acta*, 1840 (2), 931-934.

Giacco F, Brownlee M (2010). Oxidative stress and diabetic complications. *Circulation Research*, 107(9), 1058-1070.

Gray AM, Flatt PR (1999). Insulin-secreting activity of the traditional antidiabetic plant *Viscum album* (mistletoe). *J Endocrinol*, 160 (3), 409-414.

Gupta MM, Chari S (2005). Lipid peroxidation and antioxidant status in patients with diabetic retinopathy. *Indian J Physiol Pharmacol*, 49 (2), 187.

Hunt JV, Smith CC, Wolff SP (1990). Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. *Diabetes*, 39(11), 1420-1424.

International Diabetes Federation (2015). Diabetes Atlas: Update of mortality attributable to diabetes for the IDF Diabetes Atlas: Estimates for the year 2013. *Diabetes Res Clin Pract,* 109 (3), 461-465.

Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK, (2006). Antidiabetic agents from medicinal plants. *Curr Med Chem*, 13, 10, 1203-1218.

Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra, J (1998). Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clinical Science,* 94, 623-632.

Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto KY, Matsuoka TA, Yamasaki Y (2007). Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxid Redox Signal*, 9(3), 355-366.

Karabay G, Zağyapan R, Take G (2006). An ultrastructural study of effects of streptozotocin induced diabetes on rats peripheral nerves. *Journal of Uludağ University Medical Faculty*, 32 (3), 77-81.

Kollias AN, Ulbig MW (2010). Diabetic retinopathy: early diagnosis and effective treatment. *Dtsch Arztebl Int*, 107(5), 75-83.

Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, Shima K (2002). Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract*, 55 (1), 65-85.

Liu KW, Dai LK, Jean W (2006). Metformin-related vitamin B12 deficiency. *Age and Ageing*, 35(2), 200-201.

Malviya N, Jain S, Malviya SAP (2010). Antidiabetic potential of medicinal plants. *Acta Pol Pharm,* 67(2), 113-118.

Miller AG (1982). *Viscum album L.* Flora of Turkey and the East Aegean Islands. *Edinburgh University Press,* 7, 547.

Orhan DD, Aslan M, Sendogdu N, Ergun F, Yesilada E (2005). Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* subspecies in streptozotocin-diabetic rats. *J ethnopharm,* 98 (1), 95-102.

Öztabak KÖ (2005). The Anti-Carcinogen Activities of Lectins and Viscum Album Agglutinin (VAA). *Erciyes Üniv Vet Fak Derg,* 2, 55-59.

Simmons RA (2006). Developmental origins of diabetes: the role of oxidative stress. *Free Radic Biol Med*, 40(6), 917-922.

Simsek I, Aytekin F, Yesilada E, Yildirimli S (2004). An ethnobotanical survey of the Beypazari, Ayas, and Gudul district towns of Ankara Province (Turkey). *Economic Botany,* 58, 4.

Sone H, Tanaka S, Tanaka S, Iimuro S, Ishibashi S, Oikawa S, Yamada N (2012). Comparison of various lipid variables as predictors of coronary heart disease in japanese men and women with type 2 diabetes sub analysis of the Japan diabetes complications study. *Diabetes Care*, 35 (5), 1150-1157.

Swanston-Flatt SK, Day C, Bailey, CJ, Flatt PR (1989). Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice. *Acta Diabetol Lat,* 26(1), 51-55.

Tiwari BK, Pandey KB, Abidi AB, Rizvi SI (2013). Markers of oxidative stress during diabetes mellitus. *Journal of Biomarkers*, 10, 1155, 8-17.

Uchigata Y, Yamamoto H, Kawamura A, & Okamoto H (1982). Protection by superoxide dismutase, catalase, and poly synthetase inhibitors against alloxan-and streptozotocin-induced islet DNA strand breaks and against the inhibition of proinsulin synthesis. *J Biol Chem*, 257(11) 6084-6088.

Weiss RB (1982). Streptozotocin: a review of it pharmacology, efficacy and toxicity. *Cancer Treat Rep,* 66, 427-438.

Widatalla A, Mahadi SE, Shawer M, Elsayem H, Ahmed M (2009). Implementation of diabetic foot ulcer classification system for research purposes to predict lower extremity amputation. J Diabetes Dev Ctries, 29 (1), 1.

World Health Organisation (2014). About diabetes. World Health Organization: Retrieved April 4, 2014.

Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben, RG (2003). Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB Journal*, 17 (3), 509-511.