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The Effects of Grape Seed Extract on The Some Enzymes and Metabolites in Diabetic Rats

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ABSTRACT In this study, the effects of Grape Seed Extract (GSE) on glucose, enzymatic activities of elastase and collagenase, and certain biochemical parameters, were investigated. 32 female Wistar albino rats were used in the process. The subjects were randomly divided into 4 groups (control, diabetic (DM), GSE, DM+GSE). A single dose of saline was injected intraperitoneally to the rats in the control group and a single dose of streptozotocin (STZ, 45 mg/kg) was administered intraperitoneally to the rats in the diabetic group. In the GSE group, GSE (0.6 ml/rat) was administered daily via intragastric tube for 20 days, and DM+GSE group received a single dose of STZ intraperitoneally along with 0.6 ml GSE/rat. After 72 hours, blood samples were taken from all the rats. Rats with a fasting blood glucose level of 250 mg/dL and more were considered to be diabetic. At the end of the experiment, blood samples were taken again and were sent for elastase, collagenase, glucose, urea, total protein, cholesterol, and AOPP analysis. Glucose levels in diabetic rats were 671 mg/dL, compared to the glucose level of 335 mg/dL in the DM+GSE group. Total protein levels decreased in the DM+GSE group; the levels of glucose, urea, cholesterol and ascorbic acid also demonstrated a significant difference ($p<0.001$). No significant change was detected in the AOPP levels. The activities of collagenase ($p<0.01$) and elastase ($p<0.05$) in the DM group had moderate increases with respect to the control. It can, therefore, be surmised that the GSE reduces glucose levels, inhibits production of urea, regulates renal function, prevents possible nephropathy, overcomes albuminuria and alleviates protein loss, increases vitamin C levels and minimally affects collagenase and elastase activities.

Keywords: *AOPP, Diabetes Mellitus, Collagenase, Elastase, Grape seed extract*

ÖZ

Diyabetli Ratlarda Üzüm Çekirdeği Ekstraktının Bazı Enzim ve Metabolitler Üzerine Etkisi*

Üzüm çekirdeği ekstraktının diyabetli ratlarda kan glukozu, enzim aktiviteleri ve bazı biyokimyasal parametrelerdeki değişimlerin üzerine etkisi araştırılmıştır. Çalışmada 32 adet dişi Wistar cinsi Albino rat rastgele seçilerek eşit olarak 4 gruba ayrıldı. Kontrol grubunda (Grup I) intraperitoneal (i.p) yoldan 45 mg/kg tek doz serum fizyolojik enjekte edildi, diyabet grubunda (Grup II) ratlara 45 mg/kg tek doz streptozotosin (STZ) i.p. yoldan uygulandı, üzüm çekirdeği ekstraktı grubunda (Grup III) üzüm çekirdeği ekstraktı intragastrik tüp ile ağız yoluyla 20 gün boyunca her gün verildi (0.6 ml/rat), diyabet+üzüm çekirdeği ekstraktı grubunda (Grup IV) 45 mg/kg tek doz STZ i.p. yoldan uygulandı. 72 saat sonra açlık kan glukozu 250 mg/dL üzerinde olan ratlar diyabetli kabul edilip üzüm çekirdeği ekstraktı 20 gün boyunca verildi. Deneme sonunda ratların kalplerinden kan örnekleri alınıp elastaz, kollajenaz, glukoz, üre, total protein, kolesterol ve AOPP analizinde kullanıldı. Glukoz düzeyi diyabetlilerde 671 mg/dL iken diyabet+üzüm çekirdeği ekstraktı grubunda 335 mg/dL'ye düştü. Üre, total protein düzeyleri diyabet+üzüm çekirdeği ekstraktı gruplarında azaldı. Grupların glukoz, üre, kolesterol, total protein ve askorbik asit düzeyleri ortalamaları arasındaki fark önemli iken ($p<0.001$), AOPP değişiminde önemli fark saptanamadı. Kollajenaz ($p<0.01$) ve elastaz ($p<0.05$) aktivitelerinde diyabetli grupta kontrole göre hafif düzeyde yükseliş gözlendi. Üzüm çekirdeği ekstraktının glukoz düzeylerini azalttığını, üre artışını engelleyerek böbrek fonksiyonlarını düzenlediğini, olası bir nefropatiyi bertaraf edebileceğini, diyabetteki albuminüri ve farklı yollarla oluşan protein kayıplarını tolere ettiğini, kolesterol değişiminin olduğunu ve vitamin C düzeylerini artırdığını, kollajenaz ve elastaz aktivitelerini minimal düzeyde etkilediğini söylemek mümkündür.

Anahtar Kelimeler: *AOPP, Diabetes mellitus, Elastaz, Kollajenaz, Üzüm çekirdeği ekstraktı*

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** This study was summarized from PhD thesis of first author*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a deficiency in insulin secretion and resistance to metabolic effects of insulin in target tissues (Walter et al. 1991; Seghrouchni et al. 2002); and is one of the most common genetic etiologies of our time, presenting high morbidity and mortality rates. Disease-induced glucose causes severely dysregulated blood sugar levels in most body systems including blood vessels and nerves, leading to an increase in blood glucose levels (Phillips et al. 2004). In diabetic cases, oxidation of proteins increases in addition to lipid oxygenation, especially of extracellular proteins that are most commonly observed in collagen, elastin and myelin sheath tissues. Diabetic complications such as cataract, microangiopathy, atherosclerosis, and nephropathy develop in the lens tissues, vascular tissues and the basal membrane. (Altan et al. 2006). Many active substances and their commercial preparations of plant-derived groups of different chemical compounds can be used in the treatment of diabetes (Witters 2001). Medicinal plants that were traditionally utilized for their antioxidant properties can be processed to yield antioxidant vitamins, phenols or tannins. Phenols, especially flavonoids, were shown to exhibit antioxidant activity (Rice-Evans 1995; Abu-Amsha et al. 1996; Cao et al. 1997).

Catechin, epicatechin, quercetin were detected in the grape seed extract (Takahashi and Koboyashi 2003). Catechin, (catechin, ferulic acid, and vanillic acid), phenolic acids (p-kumaric, cinnamic, caffeic, genticidal, ferulic acid and vanilic acid), trihydroxy stilbenes (resveratrol and polydatin) were also detected. Oligomeric and polymeric flavan-3-ol units that are composed of epicatechin and their galaxies are called proanthocyanins 7 can also be found in the grape seed extract (Plumb et al. 1998). Depending on their concentration, proanthocyanidins can inhibit the production of free oxygen radicals. Among the various medicinal, therapeutical and pharmacological effects of proanthocyanidins discovered to date are its vasodilatory, anti-carcinogenic, anti-allergic, anti-inflammatory, antifungal, anti-arthritic, antibacterial, cardioprotective, immunostimulating, and antiviral effects (Özel 2006).

Proanthocyanidins inhibit phospholipase A2, cyclooxygenase and lipoxygenase enzymes involved in inflammatory reactions as well as protein kinase-C (PKC), which plays a role in intracellular signaling; angiotensin-converting enzyme (ACE), which plays a role in the formation of hypertension and the hyaluronidase and collagenase enzyme activities. Proanthocyanidins involve free oxygen radicals in highly deoxygenating reactions to impair lipid peroxidation; which serves to limit skin aging and the decline in mental functions caused by ultraviolet radiation (Özel 2006).

In this study, the main objective was to investigate the effects of grape seed extract on the changes in enzyme activities such as elastase and collagenase and the other biochemical processes that bring about diabetes.

MATERIALS and METHODS

Animal material

The animals used in the study were obtained from the Experimental Animal Unit Center of Y.Y. University. Thirty-two female Wistar Albino rats, approximately 7-8 weeks old, were used in the study. The rats were housed in cages that had a constant supply of food and fresh water at a temperature of 22 ± 2 °C and had 12-hour dark/light intervals each day during the 20-day test period. The study was conducted with the confirmation of the Board of Ethics Committee of Yüzüncü Yıl University Animal Experiments on 05.09.2013 and confirmation number 2013-09. The subjects were randomly selected and divided into 4 groups. The groups were formed in the following fashion:

1) The control group (C): The group consisted of eight rats. The blood sugar levels of female rats were measured prior to the test. Intraperitoneal (i.p) injection of 45 mg/kg single dose of saline was administered.

2) The diabetic group - not administered grape seed extract (DM): The group consisted of eight rats. The blood sugar levels of the rats were measured prior to the test. A single injection containing 45 mg/kg STZ was administered to induce diabetes in the rats. It was dissolved in cold citrate buffer at the pH of 4.5 STZ (Sigma, USA) and administered intraperitoneally (Karabay et al. 2006). Glucose levels in the blood samples taken from the tail veins were determined by the Levers Chek-TD-4222 biosensor glucometer after a 72-hour interval. Rats with blood glucose levels above 250 mg/dl were considered diabetic and were included in the study.

3) The nondiabetic group administered grape seed extract (GSE): The group consisted of eight rats. Grape seed extracts of 100 mg-SOLGAR were kept in 0.01 g/ml carboxymethyl cellulose, vortexed and intragastrically given every day for 20 days. (0.6 ml/rat) (Chis et al. 2009).

4) The diabetic group administered grape seed extract (DM+GSE): The group consisted of eight rats. Grape seed extract (100 mg-SOLGAR) in 0.01 g/ml carboxymethyl cellulose administered intragastrically every day for 20 days (0.6 ml/rat) to the rats diagnosed with DM, same as the rats in the 2nd group which had blood glucose levels above 250 mg/dl (Chis et al. 2009).

At the end of the trial i.p, intracardiac blood samples were obtained from the rats under general anesthesia with ketamine HCl (0.1 ml/100 g body weight) in plain and EDTA tubes, centrifuged at 3000 rpm for 10 minutes. The plasma components were extricated for AOPP analysis. The samples were stored at -18 °C in deep freezing until the assay was done. The serum samples were studied for elastase, collagenase, glucose, urea, total protein, and cholesterol via an autoanalyzer Roche Hitachi Cobas Integra 800 autoanalyzer.

Statistical analysis

A statistical package program was utilized to evaluate the raw data. The Kruskal-Wallis test was used for the comparison of the groups with no normal distribution, and Mann-Whitney U test was used for the group that caused the difference. Spearman correlation analysis was used to compare pairs of quantitative data. The results were assessed at a 95% confidence interval, at a significance level of $p<0.05$, $p<0.01$ and $p<0.001$, respectively (Düzgüneş et al. 1983).

RESULTS

The data obtained from the female Wistar Albino rats are presented in Table 1. At the end of the study, the difference between the groups' mean Glucose, urea, cholesterol, total protein, ascorbic acid ($p<0.001$), collagenase ($p<0.01$) and elastase ($p<0.05$) levels was found to be significant. The difference between the groups' mean AOPP levels was statistically insignificant ($p<0.05$) (Table 1)..

Table1. Glucose, urea, cholesterol, total protein, ascorbic acid, AOPP, collagenase and elastase levels of rats all groups.

Parameters	Control (C)	Diabetes (DM)	Grape Seed Extract (GSE)	Diabetes + Grape Seed Extract (DM+GSE)	p
	$X \pm S_X$	$X \pm S_X$	$X \pm S_X$	$X \pm S_X$	
Glucose (mg/dl)	123.25±5.34^{d}	671.25±39.41^{a}	150.13±14.94^{c}	335.75±23.15^{b}	p<0.001
Urea (mg/dl)	38.00±5.78^{c,d}	95.13±17.80^{a}	36.63±4.81^{c,d}	74.25±12.67^{b}	p<0.001
Cholesterol (mg/dl)	79.75±15.93^{a}	50.63±9.37^{d}	73.38±9.520 ^{b}	56.38±7.5^{c}	p<0.001
Total Protein (g/dl)	7.21±0.49^{a}	5.98±0.27^{d}	6.89±0.31^{b,c}	6.76±0.66^{b,c}	p<0.001
Ascorbic Acid (mg/dl)	2.04±0.25^{c}	1.56±0.35^{d}	2.46±0.78^{b}	2.52±0.64^{b}	p<0.001
AOPP (µmol/l)	1190.67±28.04	1088.66±164.23	1203.88±32.77	1184.49±28.17	0.101
Collagenase (ng/ml)	218.12±2.72^{c}	219.96±3.99^{b,c}	220.33±5.07^{b}	244.00±48.68^{a}	p<0.01
Elastase (ng/ml)	1.09±0.02^{c}	1.10±0.02^{c}	1.22±0.30 ^{a}	1.13±0.04^{b}	p<0.05

a,b,c.d: Different letters on the same line show that the difference between group means are statistically significant.

DISCUSSION AND CONCLUSION

Diabetes Mellitus is an endocrine and metabolic disorder characterized by impaired carbohydrate, lipid, and protein metabolism, caused by insulin dependence or insulin resistance. During the illness, specific complications such as retinopathy, nephropathy, neuropathy, and atherosclerosis may develop, and many people lose their lives because of these complications.

Okudan et al. (2011) investigated parameters such as MDA and SOD in plasma by giving a 100 mg/kg grape extract for 6 weeks to rats that were diabetized with STZ in order to understand the effect of DM on vascular complications. As a result, grape seed extract was shown to reduce oxidative stress and, consequently, vascular disease risk through inhibition of lipid peroxidation and of the changes in endothelial functioning.

Mansouri et al. (2011) measured the activities of enzymes such as albumin and superoxide dismutase, catalase, and glutathione peroxidase, which produced diabetes with 50 mg/kg STZ and were excreted in the 24-hour urine in a similar study. The diabetic group received 500 mg/kg grape seed extract for 6 weeks. They reported a decrease in lipid peroxidation and albuminuria in the group receiving grape seed extract and that the grape seed extract decreased oxidative stress and increased renal antioxidant enzyme activities and thus prevented the development of diabetic nephropathy.

Majeed et al. (2008) reported that administration of 20 mg grape seed extract to STZ-diabetic rats (weighed 250 gr) for 30 days resulted in a decrease in glucose level and liver enzyme activity. They also reported an increase in the glutathione level and that it was the antihyperglycemic effect of grape seed extract. The same researchers have found that compounds such as quercetin and resveratrol in the grape seed extract augment the cellular antioxidant defenses and prevent the negative effects of free radicals.

Streptozotocin is widely used to develop experimental diabetes. In the present study, blood glucose levels measured at 72 hours after STZ administration in the diabetic group were found to be significantly higher than that of the control group (p<0.001). This finding supports the success of the diabetes model and suggests that a dose of 45 mg/kg STZ is sufficient to produce diabetes. At the end of the 20-day treatment period after the development of diabetes, the blood glucose levels of the rats in the diabetic group and DM+GSE group were found to be significantly higher than those in the control group, and the blood glucose levels of the rats in the diabetic group were observed to be about 2 times higher than that of those in the group of DM+GSE. Glucose levels were found to be 123.25 mg/dl in the control group and 671.25 mg/dl in the DM group. In rats given grape seed extract, blood glucose level was measured to be 150.13 mg/dL, which is close to the levels in the control group. The most striking finding of the study was that the blood glucose level of 675.25 mg/dL in diabetic rats decreased to 335.75 mg/dL in DM+GSE. Observing a reduction of 50% within 20 days is promising for the treatment of a metabolic disease with major complications such as diabetes. Findings obtained in the study are in concert with other studies and it was determined that the polyphenolic compounds in the grape kernel compound have hypoglycemic effects.

Albuminuria, a marker of glomerular impairment, accelerates the deterioration of the tubular walls. Because of this, albuminuria is considered to be a risk factor for cardiovascular diseases (Araki et al. 2007). In the study of Dönmez (2008), when the results of the urea levels were compared, a significant increase was observed in the diabetic group (57.8 mg/dl) compared to the control group (33.8 mg/dl) with a value of p<0.04. Glucose level was found to be 146 mg/dl in the healthy group and 514 mg/dl in the diabetic group, indicating a significant increase in p<0.04 level.

In this study, it was found that urea levels increased in the DM and DM+GSE roups and similar values were found in the control group and GSE groups. Urea values were 38 mg/dl in the control group and 95.13 mg/dl in the DM group. A partial reduction of 20% was observed in the group given the GSE. The idea is that the urea increase in the DM group is due to dehydration resulting from an increase in the urine volume (Mert 1996). The significant increase in creatinine in the DM group compared to the control group suggests that albuminuria increases in a functional and structural level in addition to its increase in the cellular level. In addition, a significant increase in urea levels has been reported to be caused by a renal failure that reaches clinically overt nephropathy. The increase in urea, which may be associated with weight loss and dehydration in rats, is higher than the increase in creatinine, suggesting that hyperglycemia-related changes

impair renal function. As a result, DM-induced groups showed an increase in glomerular filtration rate and this increase was clinically significant, indicating that there was a hemodynamic change leading to an increase in glomerular pressure.

In a study where experimental diabetes was formed in rats, triglyceride levels were found to be higher in diabetes and other groups than in the control group ($p<0.001$). Total cholesterol levels were lower in the control group than in the diabetic group ($p<0.05$) (Yeğin 2012). In another study comparing oxidant/antioxidant status before and after diabetes, the mean HDL level in the diabetic group was significantly lower than that of the control group ($p<0.05$) and the mean cholesterol, triglyceride, LDL, and VLDL values were higher in the diabetic group than that of the control group ($p<0.05$) (Akkaya and Çelik 2010). Some studies have shown that plasma lipids and lipoproteins were at normal levels in Type 1 diabetes. Normally the rate of production/ destruction increases when HDL is consumed, and it is not possible to transport cholesterol from tissues back to the liver, and for this reason, cholesterol may increase. However, in one study, it was reported that HDL levels in patients with Type 1 diabetes could be unchanged or increased (Dullaart 1995).

In this study, it was again found that the cholesterol levels showed a decrease in the diabetic group compared to the control group in contrast to the findings in the literature. The cholesterol levels were slightly elevated in the DM+GSE group. However, in general, a decrease in cholesterol level was detected. If Dullaart (1995)'s interpretation is applied to this study, it can be said that the researcher could explain the decrease in cholesterol by observing the change in the increase in HDL levels. In another study of rabbits with diabetes mellitus, there was no statistically significant difference in the total plasma cholesterol levels between control, resveratrol and diabetes groups. In the treatment groups, there is an increase compared to the diabetic group (Tufan 2008). Increased cholesterol that causes subsequent cardiovascular problems is more common in chronic diabetic cases. In the present study, low cholesterol in the DM group may confirm that chronic complications have not yet been reached.

Dönmez (2008) found that compared to the total protein level of 6.01 g/dl found in healthy rats, this value was 6.32 g/dl in the diabetic group. Although this researcher found the total protein values to be high, in our study the total protein level of the control group was 7.21 g/dl while it decreased to 5.98 g/dl in the diabetic group. In the fourth group in which grape seed extract was given, it was found that the level of protein in the diabetic group increased from 5.98 g/dl to 6.76 g/dl.

Here, it was determined that oxidative damage to proteins caused by the oxidant effect of diabetes, and the lack of protein formed due to complications, the influence of the grape seed extract was improved by antioxidant and antihyperglycemic effects. A significant increase in creatinine and in albuminuria in the diabetic group compared to the control group was detected, along with a decrease in protein levels.

Some studies on antioxidant vitamins have been made in patients with type 1 diabetes, and there is no example of the change rate of these vitamins. The findings obtained from studies that do not specifically examine these parameters contradict each other. Low levels of antioxidant vitamins such as A, C, and E before diabetic treatment are due to the metabolic response to oxidative reactions, and the levels of these vitamins increase after the treatment. In order to make the defense mechanism of the body work better, vitamins should be present in appropriate doses in diabetics' daily diets (Halifeoğlu et al. 2005). It has been reported that dietary intake of recommended daily ascorbic acid is increased in patients with type 2 diabetes mellitus (Mooradian and Failla 1994).

In this study, the ascorbic acid level in the control group was 2.04 mg/dl, falling to 1.56 mg/dl in the DM group, which is parallel to the reported studies in the literature. A value close to the control group was obtained in the group given the GSE. The most noteworthy point was that vitamin C level, which is 1.56 mg/dl, increased to 2.52 mg/dl when the GSE was given. As can be seen, decreased vitamin C level was positively affected by the addition of grape seeds at relationship level of $p<0.001$ level. In other words, according to the results of this study, vitamin C deficiency, which leads to various complications in diabetics, can be elevated by different chemicals in the grape seed, creating the possibility of prevention of chronic complications of diabetes.

Since direct in vivo measurement of oxidative stress is very complicated, more practical methods are being tried. The secondary products of modified products that have undergone oxidation have gained importance with these tests. AOPP, which is defined as cross-linked proteins containing ditrosin, is produced by the interaction of hypochlorous acid and chloramines by myeloperoxidase in active neutrophils during oxidative stress. AOPP can be described as a safe parameter when measuring the oxidative modification of proteins (Piwowar et al. 2007). According to the results of the Kruskal Wallis H-Test to determine whether the averages of AOPP levels in the present study differed significantly between the groups, the difference was found to be insignificant ($p> 0.05$). Kural et al. (2011) found that AOPP and AGE levels were slightly higher in diabetic patients with or without complications compared to non-diabetic first-degree relatives, but the difference they found was not statistically significant ($p\geq0.05$).

Ramamurthy and Golub (1983) studied gingival tissue and skin samples of diabetic rats by administering streptozotocin and measured collagenase and elastase activity in extracellular tissues. In diabetics, collagenase activity is increased both in the skin and gingiva, while elastase activity increases over time. These findings show that the gingival collagenase activity is increased both in vitro and in vivo and that there is also a greater loss of collagen at the skin compared to the gingiva.

As reported above, collagenase and elastase play an active role in the formation of microvascular complications of diabetes, besides a number of factors. The recognition of collagenase and elastase enzymes and their levels through the formation of diabetes mellitus and progression through the detection of vascular damages or structural abnormalities of organs is of prognostic importance (Korthuis and Granger 1993; Verhofstad and Hendriks 1994).

In a study on rats diabetized with 200 mg/kg STZ examining how collagen synthesis occurs in the skin or intestinal fibroblasts during the diabetic state, different amounts of glucose and insulin levels were used in normal and diabetic rat sera in fibroblast cultures, and collagen synthesis was measured. It was found that increasing the glucose concentration did not affect the collagen synthesis in skin and ileum and that it caused nonspecific protein

increases in insulin. It was also found that the diabetic rat serum collagenase synthesis was less affected by deep fibroblasts than the normal rat serum. In addition, fibroblasts in ileum responded less to the diabetic sera (Verhofstad et al. 1998).

Researchers have also investigated the in vitro elastase inhibitory effect of the proanthocyanidins. The polymerized fractions of proanthocyanidins have been reported to have a high anti-elastase activity (Bos et al. 1996). Serum elastase 1 activity is reported to be elevated in chronic pancreatitis, tumors, and gastrointestinal diseases, although it is highly sensitive in cases of pancreatitis and acute pancreatitis. The activity of serum elastase 1 in the prognosis of pancreatitis does not have a clinical prescription (Pekmezci 2002).Despite the fact that proanthocyanidins in the grape seed extract are known to strengthen the downstream collagen cross-links, the effect on the collagen breakdown has been less studied. In a study of collagen-collagen degradation, biodegradation of collagen fibrils was inhibited by the addition of proanthocyanidins by the grape seed extract (Green et al. 2010).

Maffei Facino et al. (1994) reported that proanthocyanidins inhibit the activities of proteolytic enzymes non-competitively in studies of reactive oxygen species and microvascular damage. This detailed study demonstrated the molecular level at which proanthocyanidins inhibited the activity of proteolytic enzymes used in the study. Proanthocyanins have been extensively studied as antioxidants in the protection of capillary vessels, skin protection, as antioxidants in food and beverages, and in epidemiological studies as a risk-reducing agent for heart diseases (Wayne 1996; Pace-Asciak et al. 1996). Nagai (1994) reported that pancreatic elastase inhibits glomerular basement membrane thickening in diabetic animals. In animals given pancreatic elastase for 12 months (especially at 10.800 U), there was a significant decrease in albuminuria levels. Thus, the positive effect of pancreatic elastase on diabetes has been demonstrated. Polymorphonuclear elastase was tested as a diagnostic method in many diseases. Payaslı (2007) found that elastase levels increased from 75.59 ng/mL to 145.07 ng/mL in the sepsis group, which may be used in early detection of neonatal sepsis ($p<0.001$). Yılmaztepe et al. (2005) investigated the levels of fecal pancreatic elastase 1 in patients with Type 2 diabetes, and found that the level of pancreatic elastase decreased significantly in 32 patients compared to the control group, but that the decrease in elastase was not related to duration of diabetes, glycemic control and alcohol consumption.

In this presented study, the activities of collagenase and elastase enzymes are similar to the literature findings summarized above. In diabetic rats, the enzyme activities were slightly elevated compared to the control group, suggesting some damage in different tissues. The increase in enzymes in grape seed extract group is also reflected in DM+GSE group. It is possible to interpret this situation as follows: the amount of GSE given during the experiment primarily does not have an inhibitory effect on collagenase and elastase, and the elevation in the diabetic group continues. The elastase activity variations between the groups are between 0.01-0.13 ng/ml, while the changes collagenase levels are between 1.84-25.88 ng/ml. In other words, the enzymatic change in the diabetes group was found to be very positive as opposed to those found in the literature: unchanged in the 20-day period, and with minimal fluctuations when given GSE. The increase in DM+GSE group suggests that diabetes continues to have an effect on tissue damage. It is possible to think that test time of this study was not enough for the aiding effects of the grape seed extract to set in in terms of elastase activity.

As a result, it has been shown that the amount of grape seed extract used in this study has an excellent reduction effect on the normally elevated glucose levels and that it can eliminate possible nephropathies that by inhibiting the increase in urea levels. It can also balance the diabetic albuminuria and protein losses caused by divergent pathways, cholesterol, and AOPP. It is possible to say that there are no noticeable side-effects and that the grape seed extract increases Vitamin C levels, which tend to decline during diabetes. Furthermore, the grape seed extract does not have an inhibitory effect on collagenase and elastase and changes their activities only in minor fluctuations. Physicians may be advised that the use of grape seed extract in diabetic individuals, which has been found to be beneficial in this trial, may be beneficial in terms of disease course and prognosis.

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