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Laurocerasus officinalis Roem: Biochemical Parameters and Antioxidant Components in Diabetic Rat Model

S. Çiftçi Yegin, A. Güder

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ABSTRACT

Background: The aim of this study is to investigate the *in vivo* antidiabetic activity of *Laurocerasus officinalis* Roem. Because this fruit is used as antidiabetic natural products in Middle and Eastern Black Sea Reagon, Turkey.

Methods: Ascorbic acid, lycopene, β-carotene and vitamin E contents of extract were determined. Also, GSH, nitrite-nitrate, ascorbic acid, MDA, catalase and lipid-linked sialic acid experiments were performed as *in vivo* trials.

Result: The fresh and dried samples of taflan were found as rich sources of ascorbic acid (240.4 and 334.1 mg/100 g samples, respectively) and β-carotene (321.58 and 462.35 µg/100 g samples, respectively). However, lycopene and vitamin E contents in fruit not detected. The taflan has a positive effect (8.1%) on weight-loss of diabetic group (DLO). Moreover, taflan application had a pronounced effect on the blood glucose level decreasing (54.2%) after STZ application. Also, it regulated the MDA level (1.96 nmol/L) in diabetic rats according to diabetic group (2.41 nmol/L). Nitrite levels have been found as 1.92, 1.84, 1.97 and 2.02 ppm and nitrate levels as 6.91, 6.72, 9.29 and 11.46 ppm for the same samples, respectively. While ascorbic acid levels of blood serum samples were determined as 28.03, 29.47, 27.70 and 24.43 µg/mL, catalase levels were found as 0.604, 0.758, 0.479, 0.286 (mU/L), respectively. According to the obtained results, *Laurocerasus officinalis* Roem might have therapeutic antidiabetic effect, which suggest its bioactive components as candidate of natural pharmaceuticals.

Key words: Antidiabetic, Antioxidants, Enzymes, *Laurocerasus officinalis* Roem, Rat.

INTRODUCTION

Diabetes mellitus is a widespread disease in the world and it occurs by a lack of the action or secretion of insulin. The effects of diabetes mellitus may be long-term damage, dysfunction and lack of various organs (Baydaş *et al.* 2002). There are two main clinical types of diabetes mellitus, which are known as type I and II diabetes mellitus. Type I diabetes mellitus [or insulin-dependent diabetes mellitus is called (IDDM)] is an insulin-dependent autoimmune disease that is very common in human populations, regardless of gender (Öcal *et al.*, 2020).

Type I shows up at a young age and rapidly exacerbated. For treatment of type I diabetes mellitus, insulin injection is preferred as a substantial application. This metabolic disorder results from a deprivation of pancreatic β-cells so insulin production is inadequate for human body. IDDM patients must pay attention between insulin dose and nutrition quantity during length of their life. Type II [or noninsulin-dependent diabetes mellitus is called (NIDDM)] generally occurs in older age and obese people. This type symptoms are more moderate and usually unrecognizable at beginning.

The other name of type II diabetes mellitus is insulin resistant diabetes. This is indeed a group of diseases where insulin regulatory activity is defective. Insulin production is unproblematic but some properties of the insulin response system are impaired and known insulin-resistant patient. Also, scientists are still trying to explain the connection between type 2 diabetes and obesity. Both types show important typical symptoms including polydipsia and consequently consumption of excessive water, frequently micturition (polyuria). These symptoms stem from large amount glucose excretion in the urine which is called as glucosuria (Nelson and Cox, 2013).

Diabetic patients of both types cannot effectively take glucose from the blood. Because of increasing of blood glucose level, release of insulin stimulates the movement of GLUT4 into the plasma membrane. Other characteristic metabolic changes in diabetes are excessive but incomplete oxidation of fatty acids in the liver. The high ratio of [NADH]/[NAD^+], which is produced by β-oxidation, inhibits the citric acid cycle, so produced acetyl-CoA, produced by β-oxidation, which is cannot be fully oxidized by this cycle. Acetyl-CoA accumulation causes excessive production of ketone bodies such as acetoacetate and β-hydroxybutyrate which cannot be used by extrahepatic tissues as rapidly as in liver cells (Nelson and Cox, 2013).

In addition to these ketone bodies, the blood of diabetic patients also comprises acetone resulting from the self-produced decarboxylation of acetoacetate. The produced

Department of Medical Services and Techniques, Vocational High School of Health Services, Giresun University, Giresun, Turkey.

Corresponding Author: A. Güder, Department of Medical Services and Techniques, Vocational High School of Health Services, Giresun University, Giresun, Turkey. Email: aytac.guder@giresun.edu.tr

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acetone is volatile and breathing sometimes has a characteristic odor mixed with ethanol in patients with untreated diabetes. Due to high blood sugar, a diabetic person experiencing mental confusion is occasionally mistaken diagnosed as intoxicated, which may be fatal. Overproduction of ketone bodies, which is known as ketosis, causes an increase in the concentration of ketone bodies in the blood (ketonemia) and urine (ketonuria). Ketone bodies are ionized carboxylic acids that release protons. This acid production in uncontrolled diabetes affects the capacity of the bicarbonate buffering system of blood by decreasing blood pH, which causes a condition called acidosis. Ketoacidosis occurs when it is associated with ketosis, creating a life-threatening condition (Nelson and Cox, 2008).

Biochemical measurements of blood and urine samples are very important and necessary for the diagnosis and treatment of diabetes. A sensitive diagnostic criterion is provided by the glucose-tolerance test. In this test, the patient consumes a test dose of 100 g glucose dissolved in a glass of water after overnight. Blood glucose concentration is measured at 30 min or 1 h intervals prior to the test dose and for several hours. A healthy individual tolerates glucose easily and blood glucose is not more than about 9 or 10 mM; little or no glucose in the urine. In contrast to healthy person, diabetic patients tolerate the glucose test dose poorly, so blood glucose levels exceed the kidney threshold (about 10 mM) and cause glucose to appear in the urine (Nelson and Cox, 2008).

Laurocerasus officinalis Roem belongs to the Rosaceae family and is an important fruit grown mostly on the shores of the black sea region (Alasalvar *et al.* 2005). It is locally known as Taflan or Karayemiş. This fruit is widely consumed by the local people and is also used for medical purposes. It is mostly consumed as fresh fruit but it can also be used as dried, pickled, molasses, jam, marmalade and juice products. Besides its use for food, both fruit and seeds of cherry laurel are used as traditional medicines in the treatment of stomach ulcers, gastrointestinal complaints, bronchitis, eczema, long years as hemorrhoids and diuretic agents in Turkey (Güder and Korkmaz, 2012).

Different parts of this plant are used for various purposes. Its leaves are used as almond flavouring, anti-spazmodics, narcotics and sedative chemicals. In addition, its fruits and leaves are widely used in the perfumery and paint industry (Akdeniz and Gündogdu, 2007) and also this species shows good antioxidant properties (Liyana-Pathirana *et al.* 2006).

Within this study, ascorbic acid, lycopene, β-carotene and vitamin E contents of extract were determined. Also, GSH, nitrite-nitrate, ascorbic acid, MDA, catalase and lipid-linked sialic acid experiments were performed as *in vivo* trials.

MATERIALS AND METHODS

Plant materials and extraction

Taflan was collected by the corresponding author in July-August 2014 from Giresun University Güre Campus, Giresun, Turkey and the specie of fruit was authenticated by Dr. İlginç Kızılpınar Temizer, Giresun University. A voucher specimen (No. 201407010801) has been deposited in the authors' laboratory. After samples were dried in an oven at 40°C, they were chopped by a hand blender. The small pieces were subjected to extraction using Soxhlett apparatus for 24 hours, with absolute ethanol. The extracts were filtered over Whatman No.1 paper. The extracts were evaporated to dryness; the filtrates were frozen and lyophilized at -70°C and under 10 µm Hg pressure. The residues were placed in a plastic flask and kept at -30°C until used (Güder *et al.* 2014).

Animal materials

Animal materials (Wistar rats) were provided from Saki Yenilli Experimental Animals Production Laboratory Company. They were weighted between 250 and 300 g male Wistar rat and each seven animals (7-8 weeks old) were divided into four groups by selecting randomly. These groups were identified as control group (CG), diabetes group (DG), *Laurocerasus officinalis* Roem group (LO) and diabetes-*Laurocerasus officinalis* Roem group (DLO). They were been fed by using commercial rat pellets and clean drinking water *ad libitum* and kept at room temperature (approximately 25°C).

Animal treatment

The *in vivo* applications were done as following:

Control group (CG)

60 mg/kg a single dose of SF (serum physiologic) was injected intraperitoneally (i.p.).

Diabetes group (DG)

60 mg/kg streptozotocin (STZ) dissolved in cold citrate buffer (pH: 4.5) was injected as i.p. (Karabay *et al.* 2006).

Laurocerasus officinalis roem group (LO)

Laurocerasus officinalis Roem extract was dissolved in distilled water and was orally applied by gavage at the level of 50 mg/kg/day during 28 days (Rencuzogullari and Erdogan, 2007).

Diabetes-*Laurocerasus officinalis* Roem group (DLO)

60 mg/kg single dose of STZ was applied like DG. In the blood samples taken from the tail vein of rats at 72nd hours after STZ injection, glucose levels were determined by glucose meter and strips and we accepted that blood glucose level is over 250 mg/dL as patients. *Laurocerasus officinalis* Roem extract was dissolved in distilled water and was orally applied by gavage at the level of 50 mg/kg/day during 28 days. At the end of 28 days, blood samples were taken from the animals and they were disaccharified. Serum samples were obtained from blood samples by centrifuged.

GSH assay

A total of 100 µl complete blood samples, 1.8 mL of distilled water and 3 mL of distilled precipitation solution (metaphosphoric acid, EDTA, NaCl) were mixed respectively and allowed to stand for 5 min. After filtration, 2 mL of

samples and 8 ml of phosphate buffer was mixed. Then, 1 mL DTNB (40 mg DTNB was prepared in 1% sodium citrate) was added and absorbance values were recorded at 412 nm (Beutler *et al*. 1963).

Nitrite-nitrate levels assays

100 µL serum samples and 3 mL double distilled water were mixed and vortexed. After addition of 1 mL coupling reagent (85% phosphoric acid, sulfanilamide, N-1-naphthyl-ethylenediaminedihydrochloride). The mixtures were vortexed for 10 min and absorbance values were determined at 520 nm. The obtained values were used for determination of nitrite levels. For the determination of nitrate levels, 100 µL of serum samples, 1 mL of $CuSO_4$ (50 mg/L), 1 mL $(NH_2)_2SO_4$ (1 g/L), 1 mL NaOH (8g/L) and 1 mL coupling reagent were mixed respectively and vortexed. The mixture absorbances were recorded at 520 nm (Sthar, 1977).

Ascorbic acid assay

400 µL of serum samples and 2.6 mL of TCA (10%) were mixed. The sample held for 5 min and was centrifuged at 2000 rpm for 5 min. 1 mL of supernatant and 400 µL of DNPH solution was mixed, then this mixture was incubated by 3 h at 37°C. Samples were cooled in ice and 1.6 mL of sulfuric acid were added and waited for 30 min at room temperature. Absorbance values were recorded at 520 nm (Natelson, 1961).

MDA assay

A total of 200 µL of complete blood, 0.8 mL of phosphate buffer (pH = 7.4), 0.025 mL of BHT (8.8 mg/mL of absolute ethanol) and 0.5 mL of TCA (30%) were mixed respectively and stirred for 2 h at -20°C. 1 mL of the supernatant of the samples centrifuged at 2000 rpm for 15 min. 75 µL of 0.1 M Na_2-EDTA and 250 µL of TBA (1%) were mixed and waited at 90°C for 15 min. Absorbance values were recorded at 532 and 600 nm at the room temperature (Gutteridge, 1995).

Catalase assay

2 mL serum sample was mixed with 1 mL H_2O_2 (in 3.4 mL/L phosphate buffer) and absorbance values were recorded at 240 nm at 0 and 15 sec as two readings (Aebi, 1984).

Lipid-associated sialic acid (LSA) assay

44.7 µL serum was mixed with 150 µL of distilled water and vortexed for 5 seconds. Then, this mixture in ice bath was mixed with 3 mL of chloroform-methanol (2:1) mixture and vortexed for 30 seconds. 0.5 mL of distilled water was added and centrifuged at 2500 rpm for 5 min. 1 mL of supernatant and 50 µL of phosphotogenic acid (1 g/mL) were mixed and centrifuged at 2500 rpm for 5 min. After the supernatant portion was removed, 1 mL of distilled water and 1 mL of resorcinol reagent (0.02 g/mL) was added respectively and heated 15 min in the water bath. Then, this mixture was waited in water-ice bath for 10 min. 2 mL of butyl acetate-butyl alcohol (85:15) was added to this cooled, vortexed and centrifuged at 2500 rpm for 5 min, respectively. Absorbance readings were done at 580 nm against distilled water (Katopodis *et al*. 1982).

Ascorbic acid contents of extracts

The ascorbic acid contents of the extracts were determined according to the Klein and Perry method (Klein and Perry, 1982). For this reason, 100 mg of the extracts were mixed with 10 mL of 1% metaphosphoric acid on a magnetic stirrer for 45 min at room temperature *and* then filtered using by Whatman No. paper. After taking 1 mL of filtrate and 9 mL of 2, 6-dichlorophenolindophenol were mixed and absorbance values were measured at 515 nm after 30 min. The ascorbic acid contents were calculated from the standard L-ascorbic acid calibration graph plotted as standard.

Lycopene and β-carotene contents of extracts

Lycopene and β-carotene contents of the extracts were investigated according to Nagata and Yamashita method (Nagata and Yamashita, 1992). After extracting with absolute methanol, sample was evaporated to dryness. 100 mg of the extract and 10 mL of acetone-hexane (2:3) mixture was stirred vigorously for 1 min. Whatman No. 4 filter paper was used for filtration. Filtrated samples' absorbance values were measured at 453, 505, 645 and 663 nm. Lycopene and β-carotene contents of samples were calculated according to the following formulas:

$$\text{Lycopene } \frac{\text{mg}}{100 \text{ ml}} =$$

$$0.0458 \times A_{663\,nm}\ 0.204 \times A_{645\,nm} + 0.3725 \times A_{505\,nm} - 0.0806 \times A_{453\,nm}$$

$$\beta\text{-Carotene } \frac{\text{mg}}{100 \text{ ml}} =$$

$$0.206 \times A_{663\,nm} - 1.22 \times A_{645\,nm} - 0.304 \times A_{505\,nm} + 0.452 \times A_{453\,nm}$$

Vitamin E contents of extracts

The vitamin E contents of the extracts were determined according to the Martinek method (Martinek, 1964). This method is determination of the reduction of Fe^{+3} to Fe^{+2} by the effect of vitamin E based on spectrophotometric measurement of the complex formation between the Fe^{+2} and the 2,4,6-tripridyl triazin (TPTZ) reagent. For this purpose, 100 µg/mL concentration of vitamin E standard (α-tocopherol) was prepared in ethanol. 1.0 mL of absolute ethanol and 1.0 mL of the sample/standard solutions were mixed. 1.0 mL of xylene were added to the tubes and centrifuged at 4000 rpm for 10 min. 0.5 mL of 0.12% TPTZ reagent were added in the 0.5 mL of supernatant portion. After 3 min, the absorbance values of the sample tubes were read at 460 nm. The reason for not reading the absorbance values of standards at 460 nm is that the standards do not contain carotene. 0.1 mL of 0.12% $FeCl_3$ solution was added into the tubes and the absorbances were measured at 600 nm after 3 min. The content of vitamin E in the samples was calculated according to following formula:

$$\text{Vitamin E } \frac{\text{mg}}{100 \text{ mg dried extract}} = \frac{A_{Sample1} - (0.4 \times A_{Sample2})}{A_{Standard}}$$

$A_{Sample1}$: Absorbance value at 600 nm.

$A_{Sample2}$: Absorbance value at 460 nm.
A_S: Absorbance value at 600 nm.

A factor of 0.4 is the constant obtained by dividing the carotene solution at a concentration of 0.4 mg/100 mL in xylene at 600 nm by the absorbance at 460 nm.

Statistical analysis

Three parallel measurements were carried out for all test methods. Variance analysis was performed by ANOVA procedures. Significant differences of the results were investigated by Duncan's Multiple Range tests. *P* values of <0.05 were regarded as significant. The statistical results were obtained with SPSS (version 15.0.0; SPSS Inc., Chicago, IL, USA) for windows.

RESULTS AND DISCUSSION

Weight loss and blood glucose levels at the beginning and end of the experiment in diabetic-taflan (DLO) and diabetic (DG) groups were shown in Fig 1. In diabetic rats, *Laurocerasus officinalis* Roem (Taflan) fruit was used to determine the effects on some biochemical parameters. According to the data, a significant weight loss and changes in blood glucose levels were observed in the DG and DLO. When these levels are examined, the weight loss seen in DG is considerably higher than that of the DLO (approximately 2 times). Blood glucose levels were found as 9.17% and 54.2% in DG and DLO, respectively (P<0.05). Although weight loss among the clinical signs and symptoms of diabetes appears in both groups, the rate observed in the DLO is relatively moderate than DG. Moreover, when the blood glucose levels were taken into consideration, the values obtained from this group showed a significant improvement in the DLO (-54.2%). According to both weight loss and blood glucose level results, taflan extract have a positive effect for treatment of DLO.

The results of MDA, nitrite-nitrate, ascorbic acid and catalase levels obtained from the blood samples were presented in Fig 2. Free radicals, defined as compounds that have one or more non-shared electrons in their outer orbit, show reactive properties and act on many components of the cells (lipid, protein, carbohydrates and DNA *etc*). This reaction proceeds by chaining off a hydrogen atom or electron from unsaturated fatty acids. The peroxyl radicals, which are formed from reaction between conjugated dienes and oxygen, cause the chain reactions. Finally, cyclic peroxides and endoperoxides occur. Malondialdehyde (MDA) is one of the end products of lipid peroxidation and is an important indicator of lipid peroxidation (Yegin and Mert, 2013). Obtained data showed that the highest and lowest MDA levels were determined in the DG (2.41 nmol/mL) and LO (1.88 nmol/mL), respectively (P<0.05). In addition, MDA levels at the other two groups were found as 1.94 nmol/mL for CG and 1.96 nmol/mL for DLO. These results indicated that taflan could cause a significant improvement based on the MDA levels.

Depending on the oxidation state of the nitrogen element, nitrogen oxides can be found at five different molecule forms as to be NO, NO_2^-, NO_2, NO_3^- and N_2O. Nitrogen oxide (NO) is the molecule with the highest biological activity among these five nitrogen oxides and is not present as NO^- radicals in biological environments. Due to the high reactivity of NO^- radicals in aqueous systems

Table 1: Comparison of *in vivo* experimental results of all groups (P<0.05).

	GSH (mg/dL)	Nitrite (ppm)	Nitrate (ppm)	Ascorbic acid (µg/mL)	MDA (nmol/mL)	Catalase (U/mL)	LSA (mmol/L)
C	15,05	1,92	6,91	28,03	1,94	0,604	1,52
LO	15,08	1,84	6,72	29,47	1,88	0,758	1,39
DLO	15,02	1,97	9,29	27,7	1,96	0,479	1,66
DG	14,86	2,02	11,46	24,43	2,41	0,286	1,9

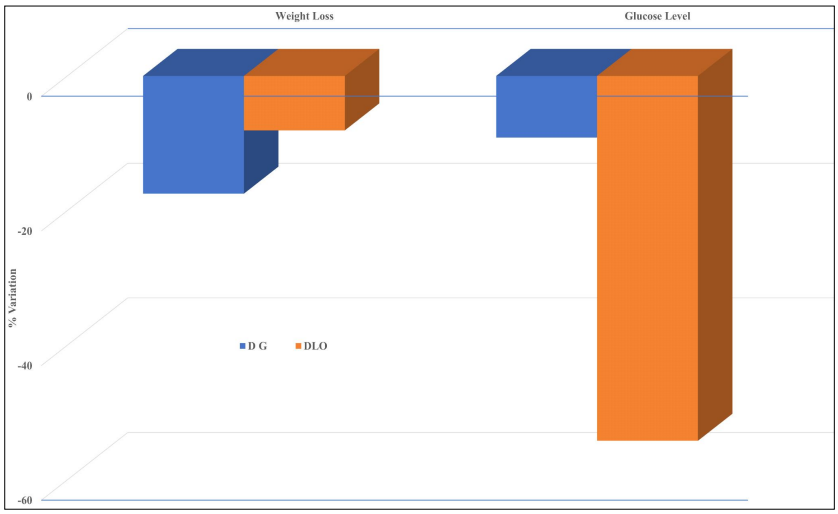

Fig 1: Comparison of weight loss (%) and blood glucose levels in diabetic-taflan (DLO) and diabetic (DG) groups.

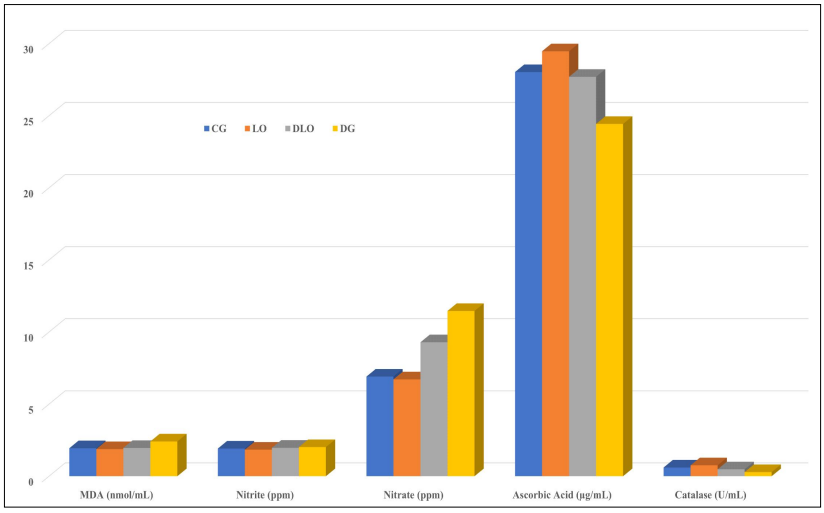

Fig 2: Comparison of MDA, nitrite-nitrate, ascorbic acid and catalase levels.

and air-liquid interface environments, it immediately converts to nitrite and nitrate products. Now days, studies that can show the relationship between DM and iNOS are generally focused on endothelial nitric oxide synthetase (eNOS) which has an effect on vasodilatation and is released from vascular endothelial cells and platelets. The cardiovascular system is the first system affected by DM *and* researchers often focus on this mechanism. NO's measurable stable end products are nitrite and nitrate, so their values can be measured in biological fluids (Pan, 2009). The highest nitrite and nitrate levels measured in our serum samples were found in DG (2.02 and 11.46 ppm), while the lowest levels were determined in LO (1.84 and 6.72 ppm) (P<0.05). Nitrite and nitrate levels in the CG and DLO were 1.92 and 6.91 ppm and 1.97 and 9.29 ppm, respectively (P<0.05). These results show that nitrite-nitrate levels are significantly increased in diabetic rats. These levels are quite low in rats given taflan.

Ascorbic acid (AA) is an antioxidant vitamin that cannot be synthesized in human body, but dissolves in blood, tissues and water phase of the cell. The antioxidant properties of ascorbic acid are enhanced in the presence of other reducing agents such as reduced glutathione (GSH) and nicotinamide adenine dinucleotide (NAD). Disruption of glutathione metabolism and decrease in AA levels are among the causes of insufficiency of antioxidant defence in diabetes. The need for AA has been shown to increase in experimental diabetic rats (Özer and Gönül, 2006). In our experience, a similar result has emerged. The highest and lowest serum levels were seen to be in the TG (29.47 µg/mL) and in the DG (24.43 µg/mL), respectively (P<0.05). Serum ascorbic acid levels in the other groups were found as 28.03 µg/mL for CG and 27.70 µg/mL for DLO (P<0.05). These results showed that ascorbic acid levels were negatively affected in the DG and there was no significant change in the treated groups. In fact, ascorbic acid levels in the healthy and LO were higher than in the CG.

Catalase is a hemoprotein consisting of four hemoglobin groups, each of which contains Fe^{+3} and is found in peroxisomes. H_2O_2 formed by superoxide dismutase (SOD) breaks down catalase peroxidases into oxygen and water. Glutathione peroxidase has a lower KM value against H_2O_2 than catalase. That is, H_2O_2 is degraded by glutathione peroxidase at low concentrations and catalase at high concentrations. Catalase activity is more intense in erythrocytes, liver and kidney. In a study of patients with type 2 diabetes, it was seen that serum catalase activity increased in patients with diabetes, which emphasized that may be due to a compensatory mechanism to protect the organism from lipid peroxidation (Memişoğulları, 2005). Within this study, catalase activity was highest in LO (0.758 U/mL) and lowest in DG (0.286 U/mL) (P<0.05). The catalase activities in the CG and DLO were found as 0.604 and 0.479 U/mL, respectively (P<0.05). These results shows that catalase activity in diabetic rats decreased considerably, particularly in LO, which indicate a positive effect of taflan on catalase activity.

Lipid-bound sialic acid (LSA) and glutathione (GSH) results obtained from the blood samples are shown in Fig 3. In normal human serum, sialic acid is available in small amounts (1-3 µmol/L), mostly due to glycoproteins or glycolipids [total sialic acid (TSA), 1.5-2.5 mmol/L]. Studies have reported a relationship between serum TSA and cardiovascular mortality in the general population. In addition, circulating sialic acid concentration was found to be elevated in type 2 diabetics when compared to healthy non-diabetic individuals. Similar studies have shown a relationship between serum sialic acid levels of type 1 and 2 diabetic patients and between retinopathy and sialic acid levels in type 2 diabetic patients (Ateş *et al.* 2009). In our diabetic groups, serum LSA levels were highest in DG (1.90 mmol/L) and lowest in LO (1.39 mmol/L) (P<0.05). The values in the CG and DLO were found as 1.52 and 1.66 mmol/L, respectively (P<0.05). According to these results, lipid-bound sialic acid levels were decreased in the diabetes group. LSA levels were determined as lower in the treated groups using taflan.

Antioxidant vitamins have an important role in regulation of insulin secretion. As a natural antioxidant, GSH and

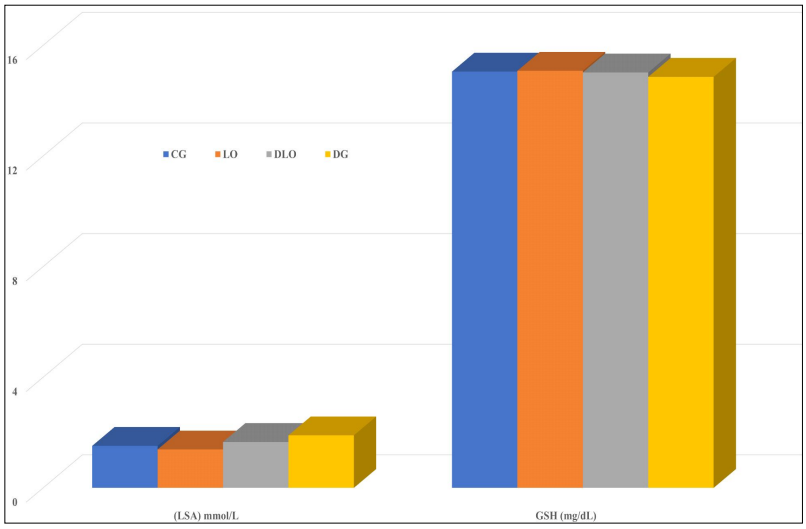

Fig 3: Comparison of lipid bound sialic acid (LSA) and glutathione (GSH) levels of samples.

Table 2: Ascorbic acid, lycopene, β-carotene and vitamin E contents of taflan fruit (fresh and dried).

	Ascorbic acid (mg/100 g)	β-carotene (mg/100 g)	Lycopene	Vitamin E
Fresh	240.37	321.58	-	-
Dried	334.09	462.35	-	-

antioxidant vitamins can prevent the formation of free radicals which are complications of diabetes. Vitamin C and glutathione are responsible for protecting of cells against free radical damage in diabetes. Many protective systems are also available. Some studies have shown that the increase in HbA1c levels in diabetes increases lipid peroxidation. Vitamin C may reduce the increased glucose levels and oxidative damage in diabetes. It has been shown that erythrocyte GSH levels decrease in diabetic patients. In addition, GSH peroxidase activity was decreased and erythrocyte lipid peroxidation was increased in diabetics. Hepatic GSH levels in diabetic patients were also found to be normal or slightly decreased (Cengiz and Cengiz, 2000). In the DG, GSH levels were lower than the other groups (14.86 mg/dL) (P<0.05). In addition, GSH levels of the CG and LO were very similar (15.05 and 15.08 mg/dL) (P<0.05). In the DLO, GSH levels were higher than the DG (15.02 mg/dL) (P<0.05). According to the results, GSH levels decreased in the diabetes group. However, it can be said that the taflan has significant protective properties in the DLO.

The amounts of ascorbic acid, lycopene, β-carotene and vitamin E of taflan fruit (fresh and dried) used in the experiment are given in Table 2. Fruits and vegetables are rich sources of vitamin A, vitamin C, vitamin E, polyphenolic compounds and flavonoids (Diplock *et al.* 1998). These compounds prevent free radical damage and reduce the risk of the chronic diseases. Therefore, the consumption of antioxidants from these sources is very useful in the prevention of cardiovascular diseases, especially atherosclerosis (Hu, 2000). Ascorbic acid, which is found in plants, animals and unicellular organisms, is either synthesized or taken up by diet. Reptiles and old birds synthesize ascorbic acid in their own kidneys (Stone, 1972). People, some other primates and guinea pigs are incapable of synthesizing ascorbic acid, so they need to get it regularly through diet (Valpuesta and Botella, 2004). L-ascorbic acid is an antioxidant, which is a powerful reducing agent that readily converts to L-dehydrocorbic acid. L-dehydrocorbic acid also has vitamin C effect. This activity is lost by hydrolysis of the lactone ring of dehydroascorbic acid to diketogulonic acid. The ascorbic acid contents of both dry and wet samples of the taflan used in the experiment were determined as 334.09 and 340.37 mg/100 g, respectively (P<0.05).

β-carotene is an organic compound and belongs to the terpenoid class. It is a strong orange-red colorant, which is abundant in fruits and vegetables. Its structure was discovered in 1930 and it is the precursor compound of vitamin A. β-carotene is converted to vitamin A in the small intestine mucosa with the help of β-carotene dioxigenase enzyme (Van Arnum, 2000). β-carotene increases the risk of lung cancer and mortality in smoking patients. These results were seen in foods containing β-carotene sold as supplements, but not in foods that naturally containing β-carotene (Russel, 2002). As ascorbic acid analysis, dry and wet β-arotene content of the samples were 462.35 and 321.58 mg/100 g, respectively (P<0.05).

Lycopene is a bright red carotenoid pigment and is found in red fruits. Lycopene is the most common carotenoid found in the human body and is one of the most powerful antioxidants (Giovannucci *et al.* 2002). Lycopene is the most powerful carotenoid that neutralizes singlet oxygen (Di Mascio *et al.* 1989). Singlet oxygen is the ROS which is responsible for skin aging and many other factors. There is evidence that frequent use of lycopene reduces the risk of cardiovascular disease, prostate cancer, cancer types, diabetes, osteoporosis and even male infertility (Bowen *et al.* 2002). The non-presecense of lycopene in both dry and wet samples was detected in this study.

Vitamin E is often found in the cell membrane and if it does not function, free radicals affect the membrane, DNA and other cell components. Vitamin E (α-tocopherol) is essential for normal reproduction, muscle functions and many other body functions. In the scientific literature, it was reported that, vitamin E reduces heart disease. It has been shown that the rate of heart disease in humans receiving vitamin E is halved (Cross *et al.* 1987). α-tocopherol terminates free radical chain reactions by transferring the phenolic hydrogen to peroxidated polyunsaturated fatty acids. The resulting oxidation product is excreted by bile secretion conjugated with glucuronic acid over the hydroxyl group on the ring. Since α-tocopherol is excreted in this way, it cannot be reused and needs to be replaced. In addition to antioxidant activity, vitamin E also affects cell proliferation through some steps in the signal transduction mechanism. In addition, vitamin E has been shown to reduce the risk of developing many heart diseases (Hudson, 1990). The most important task of vitamin E is to protect the fatty acids in membrane lipids against attacks of oxygen free radicals. Mitochondria endoplasmic reticulum and plasma membrane phospholipids have a very high affinity for α-tocopherol. Tocopherols transfer a phenolic hydrogen to the free peroxide radical in a peroxidated unsaturated fatty acid. These are compounds consisting of seven types of tocopherols, which are formed by varying the location and number of -CH_3 groups bound to the aromatic tocol ring. The order of decreasing antioxidant activities of tocopherols follows delta, beta, gamma and alpha sequence (Czinner *et al.* 1999). Vitamin E content could not be determined for dry and wet conditions of our sample.

CONCLUSION

Within this study, the health attributing effects of *Laurocerasus officinalis* Roem (Taflan) was examined through *in vivo* experiments in rats with different parameters (GSH, nitrite-nitrate, ascorbic acid, MDA, catalase and lipid bound sialic acid assays). Additionally, ascorbic acid, lycopene, β-carotene and vitamin E contents of the extracts were determined by *in vitro* analysis. Obtained results showed that taflan has a good antidiabetic activity, which suggest its use in medicinal, pharmacological and/or food industiries. It has also been shown that the taflan fruit, which is consumed by diabetic patients in different ways, is used by the people of the region for the right purpose. By isolating and characterizing the components with antidiabetic activity, *in vitro* and *in vivo* studies on these substances can also be performed.

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Declaration of interest

The authors declare that there is no conflict of interest.

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