

ANTIOXIDANT EFFECTS OF *VIBURNUM OPULUS* ON STREPTOZOTOCIN-INDUCED EXPERIMENTAL DIABETIC RATS

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ABSTRACT. Streptozotocin is a diabetogenic agent that causes a hyperglycemic situation due to oxidative stress and decreasing the capability of oxygen production properties of β -cells by creating damage on B-cells which produced insulin and located in the pancreas. The aim of this study was to investigate the effects of *Viburnum opulus* (gilaburu) on the antioxidative metabolism in experimental diabetic rats with streptozotocin. For this purpose, 40 male Wistar albino rats were divided into 4 groups of 10 rats. Following the formation of diabetes, the rats were kept in individual cages and given gilaburu oral gavage at a dose of 100 mg/kg for 15 days. At the end of the study period, MDA, SOD and CAT analyzes were performed by centrifuging the tubes from all groups with EDTA tubes. As a result of analysis, the values of CAT in blood were detected as 62,79 \pm 11,42 k/gHb; 32,10 \pm 8,81 k/gHb; 38,61 \pm 20,62 k/gHb; 21,73 \pm 21,40 k/gHb for control, diabetes, gilaburu and diabetes + gilaburu groups respectively, the values of SOD in blood were detected as 127,89 \pm 28,78 U/gHb; 168,58 \pm 41,84 U/gHb; 157,09 \pm 32,71 U/gHb; 125,27 \pm 12,13 U/gHb for control, diabetes, gilaburu and diabetes + gilaburu groups respectively, the values of CAT in blood were detected as 1,31 \pm 0,28 μ mol/L; 1,39 \pm 0,59 μ mol/L; 1,34 \pm 0,14 μ mol/L; 1,11 \pm 0,10 μ mol/L for control, diabetes, gilaburu and diabetes + gilaburu groups respectively. The results of this study support the use of gilaburu, which is widely used in Turkey, among the public, especially in alternative medicine.

Keywords: Antioxidative metabolism, diabetes, gilaburu, rat, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is characterized by the insufficiency of insulin secretion of the pancreatic gland characterized by hyperglycemia or impaired response (insulin resistance) of the tissues concerned. It is a chronic metabolic disease that negatively affects carbohydrate, protein and fat metabolism. All types of diabetes are characterized by hyperglycemia, relative or absolute absence of insulin, selective insulin resistance by the route, and the development of diabetes-specific pathology in the retina, renal glomerulus and peripheral nerve [1].

1-3% of the oxygen used by the body turns into Reactive Oxygen Species (ROS) and the resulting ROS causes oxidative stress-mediated dysfunction such as cancer, coronary vascular disease, and diabetes in the organism. There is a balance between free radicals and antioxidants in the living organism and this balance may vary depending on endogenous or exogenous factors. Oxidative stress occurs as a result of changes in favor

of free radicals. To prevent this damage caused by oxidative stress, antioxidant systems are activated and try to maintain balance [2, 3].

Antioxidants, enzymatic or nonenzymatic, provide signal transduction, immune response and regulation of proliferation under normal physiological conditions. Antioxidants may be a possible treatment alternative in the treatment and prevention of ROS-induced cancer, diabetic complications and cardiovascular diseases [4].

From ancient times to the present day, thanks to plants, protection and treatment of diseases have been made and accordingly, a rich accumulation of knowledge has emerged. One of the world's richest countries in terms of plant diversity is Turkey. Approximately 1000 plant species are used in Turkey country for treatment purposes and gilaburu (*Viburnum Opulus L.*) is one of these plants [5]. It is used as a hemostatic and external vasotonic in diuretic, laxative, antispasmodic, sedative, gynecological hemorrhages of leaves and bark of fruits of gilaburu plant against stomach pain, biliary and liver diseases and kidney stones [6].

The substances isolated from extracts made from the bark of the plant are biologically active substances such as phenolic compounds, saponins, alkaloids, triterpenes and iridoid glycosides [7]. In a study on gilaburu, it has been reported that unlike hemostatic effects, diuretic, analgesic and sedative effects, it reduces carcinogenic tumors and urinary infections [8].

The aim of this study was to investigate the effects of gilaburu on the antioxidative metabolism in experimental diabetic rats with streptozotocin (STZ).

MATERIALS AND METHODS

Materials

ELISA Device (Thermo Scientific Multiskan GO, USA) and Plate Washing Bath, Streptozotocin (Sigma, S0130), Sodium citrate (Sigma-Aldrich, C7254), Citric acid (Sigma-Aldrich, 251275), Centrifuge Device (Hettich Universal 32 R, Germany), commercial gilaburu juice.

Study Groups

Forty male Wistar albino rats were divided into 4 groups after 1 week of rest. Fasting blood sugars and body weights were measured during 1 week adaptation. Approval was obtained from Kırıkkale University Animal Experiments Local Ethics Committee (Ethics Committee no: 16/86). The research groups and the substances to be given are presented in Table 1.

After 72 hours of STZ injection, glucose measurements were performed with blood samples taken from the tail vein of rats to measure fasting blood sugar and rats were considered diabetic as 200 mg/dl [9]. At the end of the study, blood was taken from all groups into EDTA tubes and the tubes were centrifuged. Then, MDA, SOD and CAT analyzes were performed with the help of commercial kits.

SOD activity

SOD activity was performed as indicated in the commercial kit (CAYMAN, 706002). According to this; For SOD activity, samples were diluted 1: 5 with sample buffer before testing (Table 2).

Table 1. Working groups and application of streptozotocin and gilaburu to the groups.

Grups	Application
Control group	On the third day of the study, phosphate-citrate buffer (0.1 M, pH 4.5) was administered by intraperitoneal injection
Diabetes group	On day 3 of the study, 65 mg/kg STZ was dissolved in phosphate-citrate buffer (0.1 M, pH 4.5) and administered by intraperitoneal injection.
Gilaburu group	On the 6th day of the study, gilaburu was given by oral gavage at a dose of 100 mg/kg for 15 days
Gilaburu administered diabetic group	On day 3 of the study, 65 mg/kg STZ was dissolved in Phosphate-Citrate Buffer (0.1 M, Ph 4.5) and administered by intraperitoneal injection. Following diabetes, 100 mg/kg dose of gilaburu was given orally for 15 days.

Table 2. Preparation of SOD Standards

SOD Stock (μ l)	Sample Buffer (μ l)	Final SOD Activity (U/ml)
0	1.000	0
20	980	0.005
40	960	0.010
80	920	0.020
120	880	0.030
160	840	0.040
200	800	0.050

200 μ l of diluted Radical detector was added to each well. Standards (0, 0.005, 0.010, 0.020, 0.030, 0.040, 0.050 U/ml) and 10 μ l of the samples were then added respectively. To initiate the reaction, 20 μ l of xanthine oxidase was added to the wells and shaken carefully for 2 minutes. Cover the plate and incubate for 30 minutes at room temperature and read on the ELISA reader at a wavelength of 440 nm.

CAT activity

CAT activity was performed as indicated in the commercial kit (CAYMAN, 707002). According to this; 100 μ l of diluted assay buffer and 30 μ l of methanol was added to each well. Then standards (0, 5, 15, 15, 30, 45, 60, 75 μ M Formaldehyde), dilute catalase as positive control and 20 μ l of samples were added (Table 3).

Table 3. Preparation of CAT Standards

Formaldehyt (μl)	Sample Buffer (μl)	Final Concentration (μM Formaldehyde)
0	1.000	0
10	990	5
30	970	15
60	940	30
90	910	45
120	880	60
150	850	75

To initiate the reaction, 20 μl of diluted hydrogen peroxide was added to the wells, and the plate was capped and shaken with a plate shaker at room temperature for 20 minutes. 30 μl of potassium hydroxide was added to each well to terminate the reaction, and then 30 μl of Catalase chromogen was added. The plate was sealed and carefully shaken for 10 minutes at room temperature with a plate shaker. 10 μl of catalase potassium periodate was added to each well and the plate was capped and shaken for 5 minutes at room temperature with a plate shaker. It was read at ELISA reader at 540 nm wavelength.

MDA Analysis

Malondialdehyde levels in erythrocytes were determined using the Buege and Aust [10]'s method according to the procedure described for thiobarbituric acid reagents (TBARS). Optical densities were measured at 532 nm by spectrophotometer (Shimadzu UV-1700, Japan).

Histopathology

After systemic necropsy of the rats, liver and pancreas tissues were fixed in 10% buffered formalin for 24-48 hours and washed under running tap water and formalin was removed. Then, according to routine tissue follow-up procedure, graded alcohol series were passed through xylene and paraffin stations and buried in paraffin. Sections taken with a rotary microtome and 4-5 micrometer thickness were stained with hematoxylin-eosin and evaluated under a digital camera attachment trinocular research microscope (Olympus DP 25 camera and BX51 Microscope) and photomicrographs were taken.

Statistical Analysis

Statistical evaluation of the data obtained at the end of the study was performed with SPSS (15.0) statistical package program. In this context, arithmetic means, standard deviation, minimum and maximum values were determined. Repeated measures analysis of variance was used as the general linear model to determine time-dependent changes within the group (General Linear Models Repeated Measures ANOVA). Post Hoc binary comparisons were used to determine the time interval for the differences in the time-dependent differences. At this stage, Bonferroni correction was applied to adjust the confidence interval. The significance level was determined as $P < 0.05$.

RESULTS AND DISCUSSION

Forty male Wistar albino rats were divided into 4 groups. A comparison of blood glucose values between groups (mg/dl) is given in Table 4.

Table 4. Comparison of blood glucose values between groups (mg/dl). ^{a,b,c}: The difference between groups indicated by different letters on the same column is statistically significant ($P < 0,05$).

Groups	0. Day	3. Day	8. Day	12. Day	18. Day
Control group	77.6 ±2.75 75-81	84.5±5.81 ^a 79 -91	83.4±3.68 ^a 79-88	80.1±1.52 ^a 77-82	85.3±2.26 ^a 83-88
Diabetes group	83.8±5.41 73-91 ^a	336.20±42. 73 ^b 247-380 ^b	338.00±41. 94 ^c 250-381 ^b	339.80±40. 95 ^c 255-381	341.20±40. 30 ^c 260-382 ^b
Gilaburu group	82.40±4.92 74-90	81.60±5.01 ^a 73-88	81.20±4.84 ^a 72-87	81.00±4.94 ^a 72-87	80.10±4.90 ^a 72-87
Gilaburu administered diabetic group	82.50±5.21 73-90	341.60±29. 86 ^b 294-382	191.40±58. 36 ^b 99-260 ^b	181.80±50. 88 ^b 101-245 ^b	155.00±52. 85 ^b 99-246 ^b

Accordingly, there was no statistically significant difference in serum glucose levels between the control group and the gilaburu group on the 3rd, 8th, 12th, and 18th days ($p > 0.005$). On the same days, a significant increase was observed in the serum glucose levels of the experimental diabetes group compared to the control group and gilaburu group ($p < 0.005$). This was also seen in the group where experimental diabetes was established and gilaburu was given ($p < 0.005$). The increase in the experimental diabetes group and gilaburu group was less than the diabetes group ($p < 0.005$). These results showed that the gilaburu administration significantly decreased serum glucose levels in animals with diabetes ($p < 0.005$).

Lipid peroxidation and some antioxidant parameters of the groups are given in Table 5. Accordingly, catalase levels were significantly decreased in diabetes, gilaburu and Gliaburu administered diabetic groups compared to the control group ($p < 0.05$). On the other hand, there was no significant difference between the groups of diabetes, gilaburu and diabetes + gilaburu ($p > 0.05$). While there was no significant difference ($p > 0.05$) between the control, gilaburu and Gliaburu administered diabetic groups in terms of superoxide dismutase values, it was found to decrease significantly in the control and Gliaburu administered diabetic group compared to the diabetes group ($p < 0.05$). There was no significant difference between the groups in terms of malondialdehyde values ($p > 0.05$).

Table 5. Lipid peroxidation and some antioxidant parameters of groups. ^{a,b}: The difference between the groups indicated by different letters on the same line is statistically significant (P<0.05).

Parameters	Control group	Diabetes group	Gilaburu group	Gilaburu administered diabetic group
CAT (k/gHb)	62.79±11.42 ^b	32.10±8.81 ^a	38.61±20.62 ^a	21.73±21.40 ^a
SOD (U/gHb)	127.89±28.78 ^a	168.58±41.84 ^b	157.09±32.71 ^{ab}	125.27±12.13 ^a
MDA (µmol/L)	1.31±0.28	1.39±0.59	1.34±0.14	1.11±0.10

Histopathological evaluations of the groups are given in Fig. 1-4.

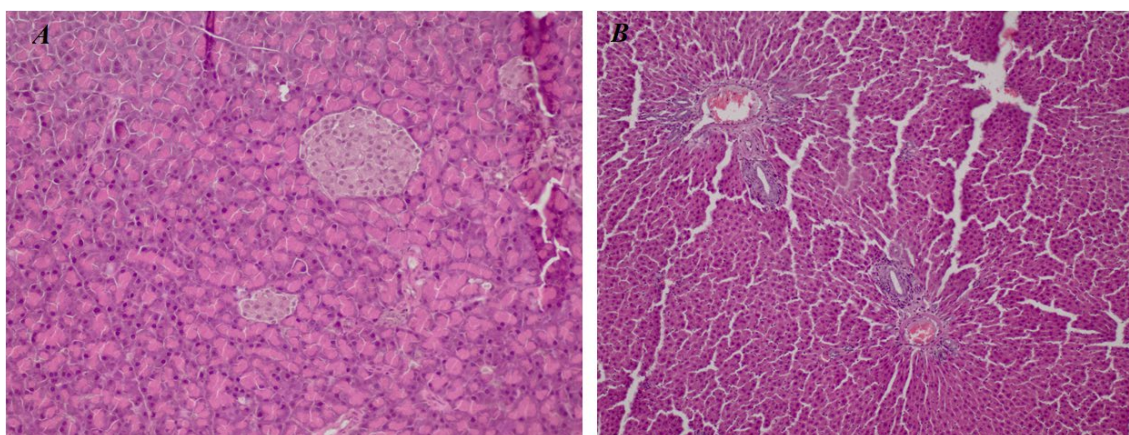


Fig. 1. Histopathological evaluation of the control group. **A:** The appearance of pancreatic interstitial islet cells, HE staining, X180, **B:** Liver normal histological appearance, HE staining, X90

In control animals, pancreatic islet cells consisted of vesicular open basophilic cytoplasmic cells with round and dense chromatin-containing nuclei, which were arranged on a narrow interstitium, and settled between the exocrine pancreas glands in solid clusters. In this group, normal liver histology was also observed in the liver.

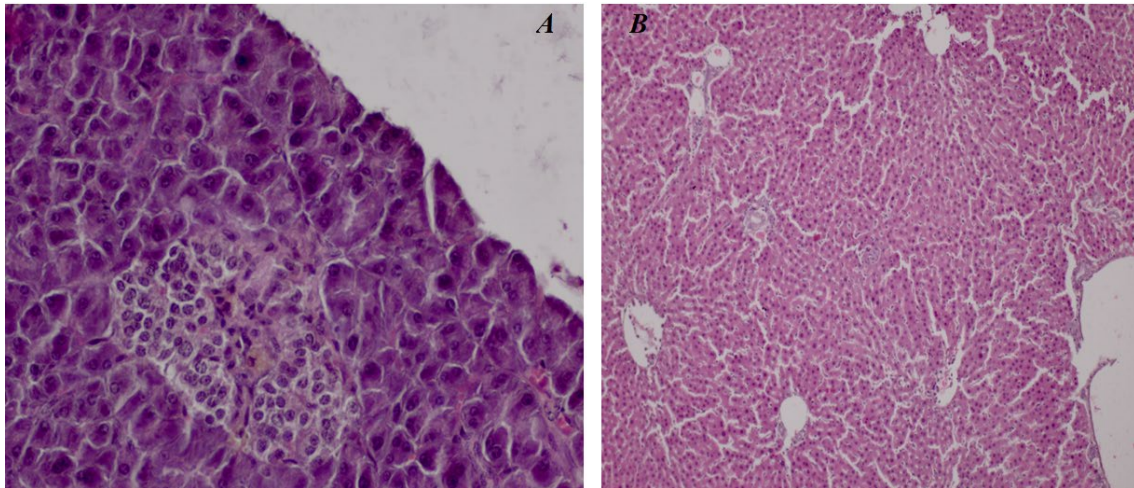


Fig. 2. Histopathological evaluation of the diabetes group. **A:** Degenerative and necrotic changes in pancreatic islet cells with interstitium edema, HE staining, X350, **B:** Mild dissociation in liver hepatocytes, HE Staining X90.

In the diabetes group; also, degenerative and necrotic changes in pancreatic islet cells decreased in general, islet cells hyperemia and edema in interstitial tissue were observed. In general, the pancreatic islets were small and atrophic. Cytoplasmic small fat vacuoles were found in the liver as well as irregularities (dissociation) in the sequence of hepatocytes.

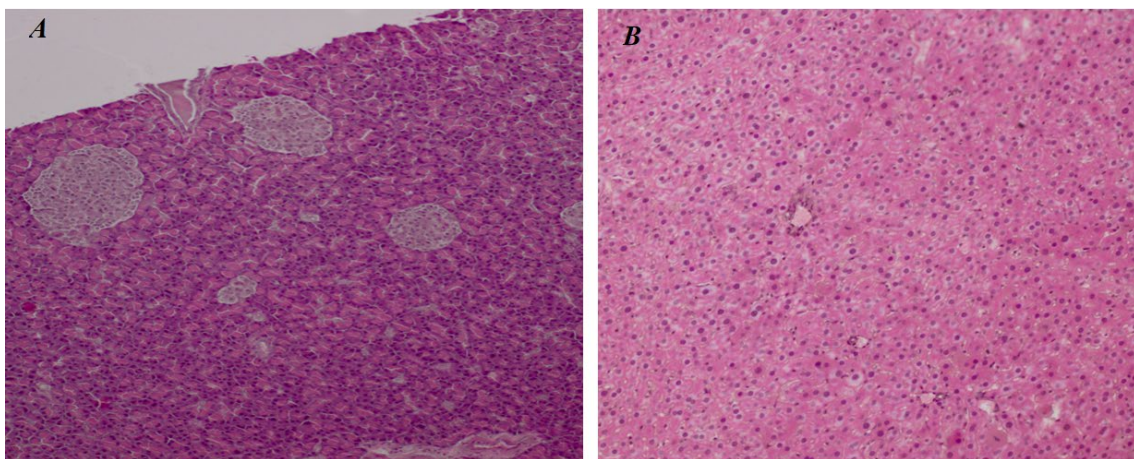


Fig. 3. Histopathological evaluation of the gilaburu group. **A:** Pancreatic islet cells, HE staining X90, **B:** Parenchymal degeneration in liver hepatocytes.

Gilaburu group animals; Pancreatic interstitial islet cells were histologically normal in size, distribution and appearance. Also, liver epithelial cells showed the clear basophilic and swollen appearance of cytoplasm, pale staining and large cytoplasmic cells were observed. Such changes are consistent with glycogenosis and/or glycogen deprivation in the liver.

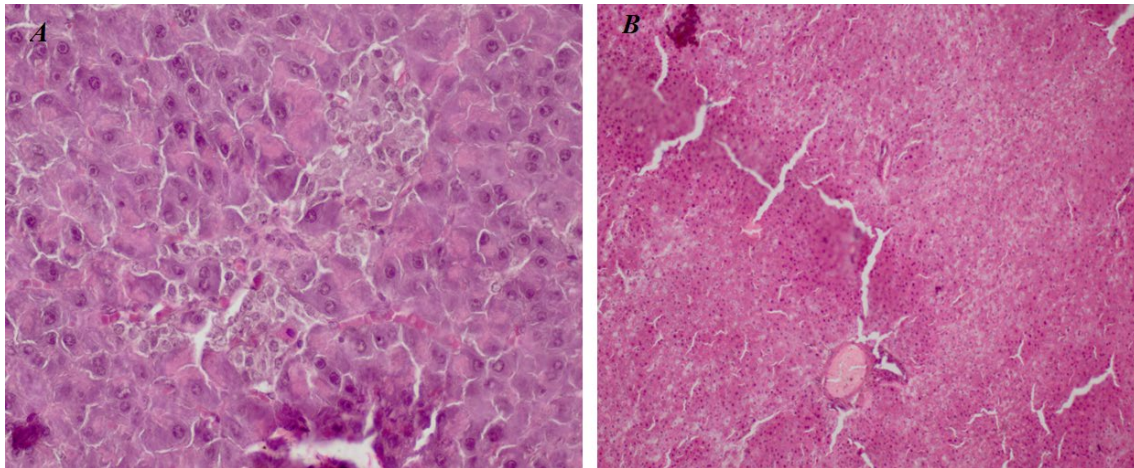


Fig. 4. Histopathological evaluation of gilaburu administered diabetic group. **A:** Degeneration and necrosis in pancreatic islet cells, HE staining, X350, **B:** Pale staining and foam cell-like appearance in the cytoplasm of liver hepatocytes, HE staining x90.

Diabetes+Gilaburu group showed severe necrosis and atrophic appearance in pancreatic islet cells and edema and bleeding in the interstitial tissue. Hepatocytes (glycogenolysis) with pale and open basophilic cytoplasm were also found in the liver.

Oxidative stress is the result of an imbalance of prooxidants and antioxidants in the organism. Antioxidants serve as a major defense against radical-mediated toxicity. Recently, attention has been paid to antioxidant compounds found in plants. In many countries, some herbal remedies are used to prevent certain diseases, depending on their capacity to remove free radicals. Gilaburu, one of the traditional drinks in Central Anatolia, is prepared from *V. opulus* fruits. The fruits are also used in traditional medicine considering their antihyperglycemic activities. The antihyperglycemic effect of *V. opulus* may be related to antioxidant activity [11].

Gilaburu contains 6.80-8.29 g gallic acid/kg polyphenol in its composition [12]. Phenolic compounds are divided into two groups as flavonoids and phenolic acids. Polyphenolic antioxidants found in natural structures of herbal teas, fruits and vegetables are flavanoids. Phenolic compounds and ascorbic acid are the major secondary metabolites with antioxidant activity [13]. Zayachkivska et al. [14] observed that Gilaburu has high antioxidant capacity and found the high phenolic compounds.

In the literature surveys, the physical and chemical properties of gilaburu fruit juice have been examined in terms of the antioxidant effect and the amount of the compounds it contains [7, 15, 16, 17, 18].

It contains high amounts of polyphenolic compounds [12, 15], L-malic acid [19] and ascorbic acid [15]. According to the data prepared for Gilaburu fruit juice, it contains a higher amount of chlorogenic acid and contains 54% of total phenolic compounds than other fruit juice and nectars. It is determined that gilaburu is the source of flavonoid (+)-catechin, (-)-epicatechin, quercetin glycosides and proanthocyanidin content [12].

As a result of the literature review, there are very few studies investigating the effect of gilaburu on antioxidant capacity in experimental diabetic rats with STZ. In a study on the protective effect of gilaburu on gastric mucosa, it was concluded that gilaburu

regulates the antioxidant activity, suppresses lipid peroxidation and increases the amount of endogenous nitric acid of proanthocyanidins [14].

In this study, it was found that gilaburu significantly decreased blood glucose levels in rats but did not decrease to normal values. In terms of clinical observations, the quality of life of the animals in the gilaburu administered diabetic group was better than in the diabetes group. It is conceivable that this effect may be due to its antioxidative effect.

The results of many studies in experimental diabetic rats and diabetic patients indicate that antioxidant system parameter levels decrease [20]. It has been reported that GSH levels in erythrocyte samples taken from diabetic patients are generally low due to increased oxidative stress and free radical content [21]. The effects of quercetin on oxidative stress in mouse liver were investigated. oxidative stress as a result of a significant reduction in GSH, CAT, SOD and GPx activities are reported to occur [22]. In this study, CAT activity showed a significant decrease in the same way as other studies, while an increase in SOD activity was observed but no change was observed in the gilaburu administered diabetic group. No change was observed in MDA values. Differences in these values suggest that the plant may be caused by a variety of antioxidant substances.

Experimental rat model studies have shown that *Viburnum opulus* prevents gastrointestinal mucosa injury by antioxidant properties and reduces MDA from oxidative stress parameters. In the study we conducted, it was observed that the amount of MDA increased again after the administration of gilaburu in diabetic rats.

Many studies [4, 20, 22, 23, 24, 25, 26, 27, 28] with experimental diabetes have shown that MDA levels are increased in diabetic groups, in parallel with increased oxidative stress and lipid peroxidation, and that MDA levels are increased in diabetic studies compared to control groups. The study we carried out was similar to these studies, and it was determined that MDA values were reduced to a normal level by giving glue to diabetic animals.

In the case of diabetes; It is stated that SOD, CAT, glutathione peroxidase, lipid peroxidation and glycemic control may have significant changes in activities. A significant effect of antioxidant capacity in cases where diabetes occurs provides an environment for the occurrence of chronic events due to oxidative stress. It is stated that antioxidants can reduce the complications of diabetes by preventing the activity of free radicals [29]. In this study; diabetes, gilaburu and gilaburu administered diabetic groups had decreased CAT and SOD values were increased in diabetes and gilaburu groups. The diabetic gilaburu group was similar to the control group. CAT values were found to be less in the gilaburu administered diabetic group than in the other groups.

CONCLUSION

The literature review did not reveal any literature on the effects of gilaburu on antioxidant levels in experimental diabetic rats. Antioxidant conflicts on gilaburu are also insufficient. Therefore, further research is needed on this subject. In recent years, scientific studies conducted in the world examined the gilaburu fruit juice antioxidant properties due to a decrease in some tumoral formations, skeletal and muscular system relaxing, vascular width regulator, high blood pressure patients in the vascular system, soothe, heart-strengthening effect, treatment against constipation and urine problems [30, 31, 32, 33] and studies are ongoing.

There are few studies investigating the effect of *Viburnum opulus* (gilaburu) on antioxidant capacity in rats with experimental diabetes with STZ. Therefore, such studies are needed. It is thought that this study will be a source for future research. Studies conducted support the use of the *Viburnum opulus* (gilaburu) plant, which is widely used in our country, among the public, especially in alternative medicine. For this reason, the importance of the use of this fruit as a medicine should be emphasized with new researches to be made on the bioactive substances found in *Viburnum opulus* (gilaburu) fruit.

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