

Antidiabetic Effect of Fig Seed Oil in Rats with Diabetes Induced by Streptozotocin

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Abstract: The anti-diabetic effect of *Ficus carica* (Fig) seed oil was investigated. 4 groups with 6 rats in each group were used in the experiment as control, diabetes (45 mg/kg streptozotocin), fig seed oil (FSO) (6 mL/kg/day/rat by gavage) and diabetes+FSO groups. Glucose, urea, creatinine, ALT, AST, GSH, AOPP and MDA analyses were done. Pancreatic tissues were examined histopathologically. When fig seed oil was given to the diabetic group, the blood glucose level decreased. In the diabetes+FSO group, serum urea, creatinine, AOPP, MDA levels and ALT and AST activities decreased statistically significantly compared to the diabetes group, while GSH levels increased significantly, histopathological, immunohistochemical, and immunofluorescent improvements were observed. It has been shown for the first time that FSO has positive effects on blood glucose level and pancreatic health. It can be said that the protective effect of fig seed oil on tissues may be due to its antioxidant activity.

Key words: fig seed oil, diabetes, rat, histopathology, glucose

1 Introduction

Diabetes; it is a metabolic disease characterized by a lack of insulin secretion and resistance to the metabolic effects of insulin in target tissues. Diabetes is accepted as one of the leading causes of morbidity and mortality in the world^{1,2)}. As a result of the disease, glucose metabolism is impaired. This causes blood sugar to rise and causes serious damage, especially to blood vessels and nerves. Many active substances obtained from different groups of chemical compounds of plant origin and their commercial preparations can be used in diabetes treatment and research. Today, it has been seen that alternative treatments are needed to control diabetes and reduce its complications. Many medicinal plants with antioxidant effects are used in the treatment and prevention of diabetes. Many active substances and their commercial preparations obtained from plant-derived groups of different chemical compounds can be used in the treatment of diabetes³⁾.

Ficus carica is referred to as fig. Ficus forms one of the largest species of angiosperms, with about 800 species of terrestrial trees, shrubs, hemiepipits (seeds sprout in the canopy, roots finally touch the ground), climbers, and reptiles that occur in tropical and subtropical climates around the world. While fresh figs contain about 12% sugar, after

drying, the sugar content rises to 50%. Dried figs, which have a special place among foodstuffs with their high caloric value, mineral and nutritional content, have a wide variety of consumption areas. *Ficus carica* is a fruit containing proteolytic enzymes, amino acids, minerals and sugars, triterpenes and organic acids. *Ficus carica* is rich in phenols, essential oils and flavonoids^{4,5)}.

Various types of figs are used in traditional medicine for their metabolic, cardiovascular, respiratory, antispasmodic, anti-inflammatory medicinal benefits. Its leaves and fruits are valued by various societies as a laxative, stimulant, cough suppressant, sedative, and mucolytic^{6,7)}. Boiling the leaves is good for hemorrhoids, while consumption of the fruit is common, especially for children, as it has laxative properties. In some recent studies, it has been reported that fig antioxidants can protect plasma lipoproteins from oxidation and provide a significant increase in plasma antioxidant capacities 4 hours after consumption⁸⁾. In a study in rats, *Ficus carica* root extract was shown to exhibit significant prophylactic protection against methanol-induced liver injury, leading to a reduction of the toxin-mediated increase in serum transaminases, ALP, and LDH. Lactate dehydrogenase level, a well-known marker for cell toxicity, was found to be significantly reduced by *Ficus carica*⁹⁾. In

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the same study, it was determined that methanol-induced oxidative stress decreased by increasing the antioxidant effects of *Ficus carica* extract. These results agree with the hypothesis that oxidative damage is neutralized when antioxidants are applied before or after induction of oxidative stress. The fact that treatment with *Ficus carica* root extract reduced increased lipid peroxidation and decreased levels of SOD, CAT and GPx indicated that fig root extract could prevent peroxidation of lipids by methanol. Moreover, these findings supported the beneficial effect of *Ficus carica* in maintaining hepatocyte integrity and function⁹⁾.

Mopuri *et al.*¹⁰⁾ in their studies in which they investigated the antioxidant, antidiabetic and antilipogenic effects of various parts of *Ficus carica* *in vitro*; they extracted *Ficus carica* fruit, leaves and root bark and measured their total polyphenol and flavonoid contents, examined the antioxidative, antidiabetic (inhibition of alpha amylase and alpha glucosidase enzymes) and antiobesogenic (antilipase) activities of the extracts. According to the results, they suggested that the fruit ethanolic extract contains high amounts of polyphenols and flavonoids (104 and 81 micrograms/mL) compared to all other extracts, and that the ethanolic extract of *Ficus carica* fruit may have potential antidiabetic and antiobesogenic substances.

Fig seeds vary greatly in size, such as large, medium or small, and their number is between 30 and 1600 per fruit^{10, 11)}. The average number of seeds per fruit is 420 and the weight of 1000 seeds is 1.28 g for "Yellow Lop" fig varieties⁵⁾. Figs have many edible seeds and are usually hollow unless pollinated. The unique nutty flavor of dried figs is also obtained from its seeds. Today, fig seeds can be used as a source of cooking oil and lubricant¹²⁾.

Ficus carica fruits contain a large number of seeds depending on their maturity and size. Linolenic acid, which also contains alpha linolenic acid (ALA), an omega-3 derivative, constitutes the largest part of the fatty acid composition of these seeds. Following linolenic acid, it contains linoleic, oleic, palmitic and to a lesser extent stearic acid, tocopherols, carotenoids, and phenolic compounds¹³⁾. *Ficus carica* seed oil is reported to contain high amounts of gamma-tocopherol, specifically 391 mg per 100 g¹⁴⁾.

Fig and its various parts have antiangiogenic¹⁵⁾, antihepatotoxic¹⁶⁾, hypolipidemic¹⁷⁾ and glycemic regulatory effects¹⁸⁾, antineoplastic effects¹⁹⁾, antibacterial and antifungal effect^{20, 21)}, antipyretic effect²²⁾, anti-inflammatory effect²³⁾ and antioxidant effect²⁴⁾ has been reported in the literature.

The aim of present study was to document the effects of oral FSO on blood glucose, some other biochemical parameters and to determine the histopathological status of pancreatic tissue in rats with STZ-induced diabetes.

2 Materials and Method

2.1 Animals and experimental procedure

This study was carried out with the approval of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (01/12/2022, 2022/12-13).

During the study, rats were obtained from Van YYU Experimental Animals Research and Application Center, fed with standard feed and kept under observation. 8-10 weeks old 24 female Wistar albino rats weighing 250-300 g were used. During the experiment, the rats were kept in cages with constant feed and fresh water in rooms with 12 hours of darkness/lighting and a temperature of $22 \pm 2^\circ\text{C}$. The animals were randomly divided into 4 groups, each consisting of 6 rats, and the experimental period was planned as 16 days. At the beginning and end of the experiment, the live weights of the rats in all groups were weighed and recorded. FSO was extracted by cold press technique and chemical composition was determined by encapsulation process. According to result the tocopherol and fatty acid results was shown as alpha tocopherol 46 mg/kg, gamma tocopherol 3918 mg/kg, delta tocopherol 76.50 mg/kg, total tocopherol 4 g/kg, linolenic (n-3) 41.1%, linoleic (n-6) 30.93%, oleic (n-9), 13%, palmitic 7.16%, stearic acids 2.90%²⁵⁾.

Working groups were organized as follows:

2.1.1 Control Group

Before the experiment, the blood sugars of the animals consisting of six rats were measured and a single dose of 45 mg/kg saline was injected intraperitoneally (i.p.).

2.1.2 Diabetes Group

Pre-experiment blood glucose levels of animals consisting of six rats were measured, 45 mg/kg single dose streptozotocin (STZ) (Sigma, USA, pH: 4.5 dissolved in cold citrate buffer), i.p. applied route^{3, 26)}. Glucose levels in blood samples taken from the tail vein after 72 hours were measured with Lever Chek-TD-4222 brand Biosensor glucometer and strips. Those with blood sugars of 250 mg/dL and above were considered as diabetic and included in the study.

2.1.3 FSO Group

The group given cold pressed fig seed oil (Biotama, TURKEY) available in the market again consisted of six rats, the blood sugars of the animals were measured before the experiment FSO was given at a dose of 6 mL/kg/day²⁷⁾ once a day for 12 days by gavage using the orogastric route at the same time of the day.

2.1.4 Diabetes + FSO Group

Pre-experimental blood glucose levels of animals consisting of six rats were measured, a single dose of 45 mg/kg streptozotocin (STZ) (Sigma, USA, pH: 4.5 dissolved in cold citrate buffer) i.p. applied route^{3, 26)}. Glucose levels in blood samples taken from the tail vein after 72 hours were measured with Lever Chek-TD-4222 brand Biosensor glucometer and strips. Those with blood sugars of 250 mg/dL and

above were considered as diabetic and included in the study. FSO was given at a dose of 6 mL/kg/day²⁷⁾ once a day for 12 days by gavage using the orogastric route at the same time of the day.

2.2 Collection of samples and biochemical analysis

After the experimental applications (16th day), 90 mg/kg ketamine i.p. was administered to all rats as an anesthetic. The thorax of the animal was shaved, cleaned with alcohol and opened with a vertical incision in the midline, then the heart was directly cannulated and a blood sample was taken. Sera obtained from blood samples were stored. Then, serum urea and creatinine levels, ALT and AST activities in sera were measured in a Roche Modüler P 800 otoanalyzer. GSH, AOPP and MDA analyses were done by ELISA as Rat Glutathione (GSH) ELISA Kit (SunRed, Cat. No: 201-11-5134), Rat Malondialdehyde (MDA) ELISA Kit (SunRed, Cat. No: 201-11-0157), Rat Advanced Oxidation Protein Products (AOPP) ELISA Kit (SunRed, Cat. No: SRB-T-88141).

2.3 Histopathological examination

Tissue specimens from salivary glands collected for histopathological examination following necropsy were fixed in 10% formalin solution for 48 h, then embedded in paraffin blocks. Sections 4 µm in thickness were taken from each block. The preparates were then stained with hematoxylin-eosin (HE) and examined under a light microscope (LeicaDM 1000, Germany). Depending on their histopathological findings, such as degeneration, necrosis and atrophy in glandular epithelium and vascular congestion, sections were graded as none (-), mild (+), moderate (++), or severe (+++). Degeneration and necrosis in glandular epithelia were defined as a degenerated or necrotic cell number 0 (-), 2-3 (+), 4-7 (++) and more than 8 (+++). Atrophy in glandular epithelia was defined as atrophic cell number 0 (-), 2-3 (+), 4-7 (++) and more than 8 (+++). Vascular congestion was classified as number of congested vessels 0 (-), 3-5 (+), 6-9 (++) and more than 10 (+++). Histopathological findings in gland tissues were scored from images obtained by digital camera from five different regions at 40x magnification²⁸⁾. All analyzes were performed blindly.

2.4 Immunohistochemical examination

Tissue sections taken on adhesive (poly-L-Lysin) slides for immunoperoxidase analysis were deparaffinized and dehydrated. Then, endogenous peroxidase was inactivated by keeping it in 3% H₂O₂ for 10 minutes. Then the tissues were boiled in 1% antigen retrieval (citrate buffer (pH;6.1) 100X) solution and left to cool at room temperature. Sections were incubated with protein block for 5 minutes to prevent nonspecific background staining in tissues. Then, primary antibody (Caspase 3) was dripped onto the tissues

and incubated in accordance with the instructions for use. Sections will be incubated with biotinized secondary antibody for 10 minutes at room temperature. Then the tissues were kept in streptavidin-peroxidase for 15 minutes. 3-3' Diaminobenzidine (DAB) chromogen will be dripped onto the sections and incubated for 5 minutes according to the chromogen uptake of the tissues. For background staining, the sections were kept in Mayer's hematoxylin for 2 minutes and then washed in running tap water. Then, the prepared sections were covered with a coverslip and examined with a light microscope (Zeiss Axio Germany). The data were expressed as nm.

2.5 Double immunofluorescence examination

Tissue sections taken on adhesive (poly-L-Lysine) slides for immunofluorescence examination were deparaffinized and dehydrated. Then, endogenous peroxidase was inactivated by keeping it in 3% H₂O₂ for 10 minutes. Then the tissues were boiled in 1% antigen retrieval (citrate buffer (pH;6.1) 100X) solution and left to cool at room temperature. Sections were incubated with protein block for 5 minutes to prevent nonspecific background staining in tissues. Then, primary antibody (8-OHdG) was dripped onto the tissues and incubated in accordance with the instructions for use. Immunofluorescence secondary antibody was used as a secondary marker (FITC Cat No: ab6785 Diluent Ratio: 1/1000 UK) and kept in the dark for 45 minutes. Then, DAPI with mounting medium (Cat no: D1306 Dilution Ratio: 1/200 UK) was dripped onto the sections and kept in the dark for 5 minutes, then the tissues were covered with a coverslip. The stained tissues were examined under a fluorescent microscope (Zeiss Axio Germany).

2.6 Statistical analysis

The SPSS 13.0 program was used for statistical analysis of the biochemical data. One-Way Anova test was used for comparison between groups and the data were evaluated with $p < 0.05$ considered significant.

SPSS 13.0 program will be used for statistical analysis in histopathological examinations and the data were evaluated with $p < 0.05$ considered significant. Duncan test was used for comparison between groups. The non-parametric Kruskal-Wallis test was used to detect group interaction, and the Mann Whitney U test was used to determine the differences between groups.

In order to determine the intensity of positive staining from the pictures obtained as a result of staining in immunohistochemical and immunofluorescent examinations; 5 random areas will be selected from each image and evaluated in the ZEISS Zen Imaging Software program. Data were statistically defined as mean and standard deviation (mean ± SD) for % area. One-Way ANOVA followed by Tukey's test was performed to compare positive immunoreactive cells and immunopositive stained areas with

healthy controls. As a result of the test, a value of $p < 0.05$ will be considered significant and the data are presented as mean $\pm$ SD.

3 Results

3.1 Biochemical findings

Twenty-four Wistar albino rats used in the study were weighed when the groups were formed and at the end of the trial period. The mean body weights of the groups and rats are shown in Table 1.

When Table 1 is examined, the initial weights were very close to each other, while the mean values (269.33-279.17 g) were determined in the control and FSO groups at the end of the experiment, while lower body weights were found in the group with diabetes group and in the group given FSO (205.40-208.20 g), respectively.

On the first day of the study, blood glucose levels were measured from the tails of all rats (first measurement). When the animals were separated according to their groups in the cages, a total of 12 individuals in the diabetes and diabetes + FSO group were injected with STZ (45 mg/kg) intraperitoneally and the fasting blood glucose levels of all groups were re-measured 72 hours later (second measurement). At the end of the experiment, blood glucose levels of all animals were measured again (final measurement) and then the anesthetic ketamine was injected. Thorax was opened and blood samples were taken directly from the heart.

Obtained data and average values were compared. Ac-

cordingly, on the first day, blood glucose levels of all animals were found to be in the range of 125-138 mg/dL in a similar way (Table 2). In order to see the effect of STZ injected to cause diabetes, blood glucose levels of all rats, which were previously placed in the experimental cages, constituting the diabetes and diabetes + FSO groups, were measured. Fasting blood glucose levels were found to be quite high in all animals in both the diabetes and diabetes + FSO groups. According to the project schedule, the application of FSO by gavage was started. On the last day of the trial fasting blood glucose levels were measured from the tails of all animals before slaughter. As shown in Table 2, the mean blood glucose levels in the diabetes group were 137.83 mg/dL at the beginning, while it increased to 511.16 mg/dL three days after STZ administration and increased to 564.20 mg/dL at the end of the 16th day. Finally, during the 16-day trial, intra- and inter-group changes in blood glucose levels of the diabetes and diabetes + FSO groups were found to be statistically significant ($p < 0.05$).

Significant change in fasting blood glucose was observed in the fourth group, namely diabetes + FSO. The level which was 125.17 mg/dL on the first day increased to 551.33 mg/dL 72 hours following STZ administration. It decreased to 484.80 mg/dL following administration of FSO. This decrease indicates a positive effect of FSO on the increase in blood sugar. Indeed, histopathological examination showed that FSO is positive for pancreatic tissue.

Serum urea and creatinine levels were high in the diabetic group. They decreased when adding FSO. An increase in ALT and AST activity was seen in the diabetic group, but an important decrease was observed with the addition of

Table 1 Live weight changes of the rats used in the study on the first and last day of the experiment.

Live weight (g)	n	Initial weights	Final weights
Control Group	6	277.00 $\pm$ 48.27 ^a	269.33 $\pm$ 55.96 ^a
FSO Group	6	272.67 $\pm$ 17.27 ^a	279.17 $\pm$ 14.77 ^a
Diabetes Group	6	246.00 $\pm$ 15.74 ^a	205.40 $\pm$ 20.62 ^b
Diabetes + FSO Group	6	257.67 $\pm$ 22.10 ^a	208.20 $\pm$ 22.160 ^b

^{a,b}: The differences between values containing different letters in the same line were found to be statistically significant at the $p \leq 0.05$ level.

Table 2 Rat blood glucose levels from 3 different measurements (1st, 4th, 16th day).

	n	Glucose (mg/dL) First	Glucose (mg/dL) Second	Glucose (mg/dL) Final
Control Group	6	138 $\pm$ 12.40 ^a	131 $\pm$ 10.23 ^a	112.17 $\pm$ 11.74 ^a
FSO Group	6	135.67 $\pm$ 13.10 ^a	127 $\pm$ 11.78 ^a	119.17 $\pm$ 11.75 ^a
Diabetes Group	6	137.83 $\pm$ 6.21 ^a	511.16 $\pm$ 68.98 ^b	564.20 $\pm$ 37.21 ^c
Diabetes + FSO Group	6	125.17 $\pm$ 16.24 ^a	551.33 $\pm$ 39.80 ^c	484.80 $\pm$ 26.13 ^b

^{a,b,c}: The differences between values containing different letters in the same line and column were found to be statistically significant at the $p \leq 0.05$ level.

FSO. In diabetes group a decrease in glutathione levels and an increase in AOPP and malondialdehyde were also detected when compared to the groups. Like some other parameters mentioned here, it changed positively and approached the amounts of the control group when FSO was given (Table 3).

3.2 Histopathological findings

3.2.1 Control Group

Pancreatic tissues were observed to have normal histological structure in the histopathological examination (Fig. 1, Photo A).

3.2.2 Diabetes Group

When the pancreatic tissues were examined histopathologically, it was determined that there was severe degener-

ation and necrosis in the cells, atrophy in the islets of Langerhans and severe hyperemia in the vessels (Fig. 1, Photo B).

3.2.3 FSO Group

In the histopathological examination of pancreatic tissues, it was observed that they were in normal histological structure (Fig. 1, Photo C).

3.2.4 Diabetes + FSO Group

In this group, pancreatic tissues were also examined histopathologically, mild degeneration in the islets of Langerhans and hyperemia in the interstitial vessels were observed (Fig. 1, Photo D). A statistically significant difference ($p < 0.05$) was found when compared to the diabetes group.

Histopathological scoring is shown in Table 4. When the

Table 3 Some serum biochemical findings of rats in four groups.

	Control Group X $\pm$ SD	FSO Group X $\pm$ SD	Diabetes Group X $\pm$ SD	Diabetes + FSO Group X $\pm$ SD
Urea (mg/dL)	33.8 $\pm$ 4.47 ^a	27.42 $\pm$ 3.28 ^a	97.02 $\pm$ 9.44 ^c	79.08 $\pm$ 12.28 ^b
Creatinine (mg/dL)	0.39 $\pm$ 0.04 ^a	0.39 $\pm$ 0.04 ^a	0.44 $\pm$ 0.06 ^b	0.40 $\pm$ 0.02 ^a
ALT (U/ L)	38.33 $\pm$ 3.50 ^a	29.50 $\pm$ 3.21 ^a	895.20 $\pm$ 236.13 ^c	540.8 $\pm$ 268.03 ^b
AST (U/ L)	107.17 $\pm$ 21.05 ^a	101.33 $\pm$ 21.86 ^a	1381.20 $\pm$ 618.48 ^c	784.20 $\pm$ 218.17 ^b
GSH (mg/ L)	210.34 $\pm$ 44.34 ^a	218.11 $\pm$ 47.21 ^a	124.67 $\pm$ 16.65 ^c	175.43 $\pm$ 25.12 ^b
AOPP (nmol/mL)	10.87 $\pm$ 2.06 ^a	11.45 $\pm$ 1.95 ^a	17.25 $\pm$ 4.31 ^b	12.36 $\pm$ 1.68 ^a
MDA (nmol/mL)	1.47 $\pm$ 0.21 ^a	1.54 $\pm$ 0.33 ^a	2.46 $\pm$ 0.74 ^b	1.8 $\pm$ 0.42 ^a

^{a,b,c}: The differences between values containing different letters in the same line were found to be statistically significant at the $p \leq 0.05$ level.

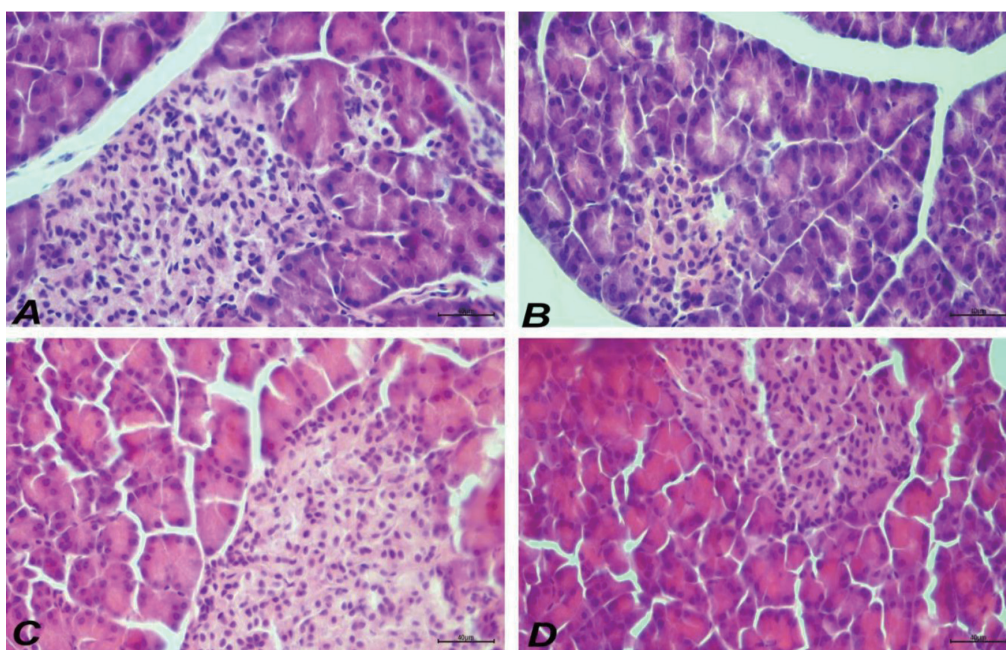


Fig. 1 Histopathological examination of pancreatic samples taken from the groups in the study (Pancreatic tissue, H&E, Bar: 40 μ m).

change in pancreatic tissue according to the groups was examined, degeneration, necrosis, atrophy and hyperemia were observed as severe with the effect of STZ in the diabetes group. While necrosis and atrophy were not observed in the diabetes + FSO group, mild degeneration and hyperemia were detected.

3.3 Immunohistochemical findings

3.3.1 Control Group

When pancreatic tissues were examined immunohistochemically, Caspase 3 expression was evaluated as negative (Fig. 2).

3.3.2 Diabetic Group

Severe cytoplasmic expression of Caspase 3 was detected by immunohistochemistry in the islets of Langerhans within the islets (Fig. 2).

3.3.3 FSO Group

Caspase 3 expression was evaluated as negative (Fig. 2).

3.3.4 Diabetes + FSO Group

Mild cytoplasmic expression of Caspase 3 in islets of Langerhans cells was demonstrated by immunohistochemistry (Fig. 2). A statistically significant difference ($p < 0.05$) was found compared to the diabetic group. Immunohistochemical results have also been summarized in Table 5.

3.4 Immunofluorescent findings

3.4.1 Control Group

When pancreatic tissues were examined by immunofluorescence method, 8-OHdG expression was evaluated as negative (Fig. 2).

3.4.2 Diabetes Group

Severe cytoplasmic expression of 8-OHdG was detected by immunofluorescence in the islets of Langerhans (Fig. 2).

3.4.3 FSO Group

8-OHdG expression was assessed as negative by immunofluorescence (Fig. 2) in pancreatic tissue.

Table 4 Scoring of histopathological findings in pancreatic tissues.

	Degeneration of islet cells	Necrosis of islet cells	Atrophy of islets of Langerhans	Hyperemia of vessels
Control Group	—	—	—	—
Diabetes Group	+++	+++	+++	+++
FSO Group	—	—	—	—
Diabetes + FSO Group	+	—	—	++

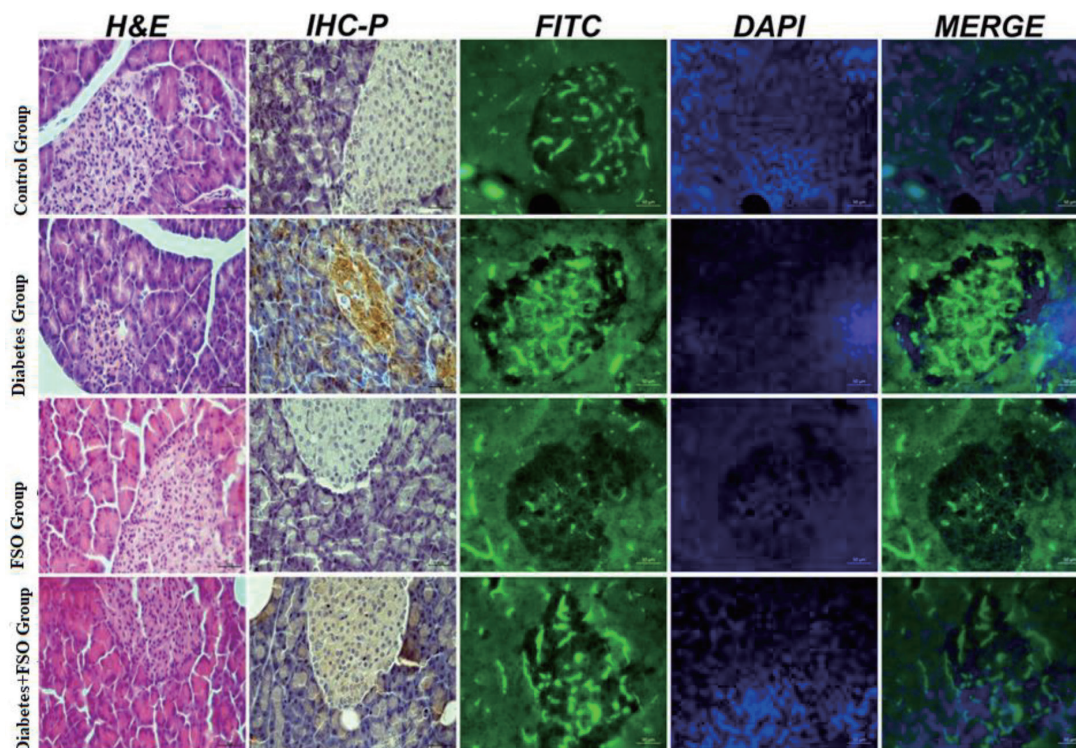


Fig. 2 Pancreatic tissue, H&E, Caspase 3 expression, IHC-P, Bar: 40 μ m, 8-OHdG expression, IF, Bar: 50 μ m.

3.4.4 Diabetes + FSO Group

Mild cytoplasmic expression of 8-OHdG was observed in the islets of Langerhans (Fig. 2) by immunofluorescence. A statistically significant difference ($p < 0.05$) was found compared to the diabetic group.

Immunofluorescent findings have been summarized in Table 5.

4 Discussion

Diabetes is a disease in which the pancreas does not produce enough insulin or cannot use it effectively and can lead to serious complications such as heart and brain damage, eye disease, kidney disease, and heart disease. Skin problems, itching, various wounds, delayed healing, frequent infections, urinary tract disease especially in women, sexual insufficiency in men, osteoporosis, and nerve damage have also been observed. Diabetes mellitus reduces the body's response and/or insulin production, glucose cannot reach the cells, the body feels hungry and produces energy, first using fat and then muscle. Since it causes the patient to lose weight. In this study, this phenomenon was observed when comparing the initial and final weights of rats and was shown to reduce body weight in diabetes (Table 1). The live body weights of diabetic rats were decreased from 246.00 to 205.40 g ($p \leq 0.05$).

It is known that various extracts prepared from the leaves, fruits and roots of fig are used in many different pathologies, including diabetes¹⁸. Indeed, it is important that the amount of unsaturated fatty acids in fig seed oil is higher than saturated fatty acids. All the oils sampled showed a significant phenolic content with varying levels of antioxidant activity. Thanks to the phenolic content of fig seed oil, it exhibits anticarcinogenic, antioxidant, antimutagenic, free radical scavenging and inhibiting lipid peroxidation properties with its high scavenging activity¹³. Fig seed oil is very beneficial for human health and controls blood vessels and other body functions. Very long chain polyunsaturated fatty acids (C18-22) and omega 3 fatty

acids are widely accepted as part of the modern diet because of their beneficial effects on metabolism. Most importantly, the protective effect of omega 3 fatty acids against cardiovascular diseases and cancer makes them more important than other dietary supplements.

Ficus carica fruits contain a large number of seeds depending on their maturity and size. Linolenic acid, which also contains alpha linolenic acid (ALA), an omega-3 derivative, constitutes the largest part of the fatty acid composition of these seeds. Linolenic acid is followed by linoleic, oleic, palmitic and to a lesser extent stearic acid. Since it contains plenty of linolenic acid in its structure, FSO is among the oils that must be taken in human health. The composition of fig seed oil is presented as: Palmitic acid % 5.0-9.0, stearic acid % 2.0-4.0, oleic acid % 14.0-24.0, linoleic acid % 20.0-35.0, α -linolenic acid % 32.0-50.0, γ -linolenic acid % 0.17, arachidic acid- up to % 0.7, eicosenoic acid % 0.5, behenic acid up to % 0.5²⁷. First of all, FSO will give strength and energy if one have fatigue, weakness, exhaustion, or difficulty getting up in the morning, enables the renewal of our cells, affects brain health, prevents vascular occlusion, arteriosclerosis, blood pressure, due to its high potassium content fig seed oil reduces the insulin amount, regulates diabetes, increases HDL cholesterol, lowers LDL cholesterol, balances triglyceride, reduces blood clotting tendency, prevents plaque formation by protecting the vascular wall, strengthen the immune system²⁹⁻³¹.

In this study, it was aimed to investigate the relationship of FSO with diabetes based on biochemical and pathological data. In the literature reviews, no study was found in this direction, that is, diabetes induced by STZ and investigating the effect of FSO on blood glucose. While there are studies that protect the liver and reduce the harmful effects of some drugs, there is no study in which the histopathological examination of the pancreatic tissue in which insulin is synthesized.

In this study, blood sugar of all rats was measured at the beginning. After forming the groups, STZ was given at a dose of 45 mg/kg to 12 rats, 6 rats in each group. Very high glucose levels were detected in these 12 rats, that is, on the 4th day, blood sugar levels reached the value of 511.16 mg/dL in the Diabetes group. When it was proven that diabetes had occurred, the fourth group, Diabetes + FSO, was given 6 mL/kg FSO for 12 days. Blood samples were taken from the hearts of all rats and their final blood sugar levels were determined. While the value of 511.16 mg/dL in the diabetes group increased to 564.20 mg/dL on the 16th day, the blood sugar level of 551.33 mg/dL in the diabetes group given FSO (Diabetes + FSO group) decreased to 484.80 mg/dL. This decrease was considered as the achievement of the project's goal. In addition, blood glucose levels in the control group and FSO group were found to be very low compared to the Diabetes and Diabetes + FSO groups, as

Table 5 Statistical data of immunohistochemical and immunofluorescent findings in pancreatic tissues.

	Caspase 3	8-OHdG
Control Group	20.42 $\pm$ 3.14 ^a	23.17 $\pm$ 4.1 ^a
Diabetes Group	81.24 $\pm$ 5.7 ^b	84.85 $\pm$ 6.2 ^b
FSO Group	21.57 $\pm$ 4.3 ^a	24.19 $\pm$ 3.4 ^a
Diabetes + FSO Group	39.81 $\pm$ 4.6 ^c	42.58 $\pm$ 5.3 ^c

^{a,b,c}: The differences between values containing different letters in the same line were found to be statistically significant at the $p \leq 0.05$ level.

expected. As a matter of fact, the difference between the blood glucose averages of this group was calculated to be statistically significant ($p \leq 0.05$).

The striking aspect of this project is that the positive effect of administering FSO to diabetic rats on blood sugar was proven by biochemical and histopathological examination of pancreatic tissue. Because the blood glucose level, which was 551.33 mg/dL, decreased to 484.80 m/dL in 12 days and the difference was statistically significant at the $p \leq 0.05$ level. Even though this difference of 66.53 mg/dL seems very small, this change within 12 days is also statistically significant.

In a study on experimental myoglobinuric acute kidney injury groups given FSO, a decrease in elevated serum urea and creatinine values without difference between high and low doses FSO an increase in tissue GSH levels in a non-dose dependent manner³²⁾. In the presented study elevated urea and creatinine levels in diabetic group decreased significantly after addition of FSO. These finding is compliance with previous studies. Biochemical values determined in this research were all summarized in **Table 3**. With these chosen parameters the health status of kidney, liver and pancreas were biochemically examined. First of all urea and creatinine levels were significantly increased in diabetes group. Addition of FSO to diabetic rats lowered obtained values. Decreases in urea and creatinine values revealed that FSO has positive effects for renal healing because of decreased values. Urea and creatinine decreased from 97.02 to 79.08 mg/dL and 0.44 to 0.40 mg/dL respectively. These decline was calculated as statistically significant ($p \leq 0.05$).

Mopuri *et al.*¹⁰⁾, investigated the antioxidant, antidiabetic and antilipogenic effects of various parts of *Ficus carica* as fruit, leaves, and root bark *in vitro* and checked their total polyphenol and flavonoid content. They examined the antioxidative, antidiabetic and antiobesogenic activities of the extracts. According to their results, fruit ethanolic extract contains higher amounts of polyphenols and flavonoids (104 and 81 micrograms/mL) compared to all other extracts. The ethanolic extract of *Ficus carica* fruit was significantly higher antioxidative, antidiabetic and antiobesogenic effects than all other extracts, indicated that the ethanolic extract of the fruit of *Ficus carica* may have potential antidiabetic and antiobesogenic agents. Serracarla *et al.*³³⁾, used the hypoglycemic effect of fig leaf decoction in Type 1 diabetic patients and in Canal *et al.*³⁴⁾ used an extract of *Ficus carica* leaves to lower cholesterol levels in diabetic rats. They thought that these pharmacological properties were probably due to the high content of phenolic compounds in these plant extracts. In the presented study similar findings with fig seed oil were obtained, here also promising antidiabetic and antioxidative effects were demonstrated.

Gond and Khadabadi¹⁶⁾ extracted the shade-dried leaves

of *Ficus carica* using petroleum ether and investigated for antihepatotoxic activity in rifampicin-treated rats. They showed hepatoprotective activity of extract by biochemical, histological examinations. A significant decrease in AST, ALT levels and a histopathological improvement were observed in the group treated with *Ficus carica* extract. The similar hepatoprotective effects were reported in this study with fig seed oil. AST and ALT activities were significantly decreased when FSO were given to STZ treated rats (**Table 3**).

In a study, Stephan Irudayaraj *et al.*³⁵⁾ reported that doses of ficus carica leaf extracts (250 and 500 mg/kg) significantly decreased blood sugar 30 and 60 minutes after administration to mice. Here, researchers suggest that insulin sensitivity is enhanced, possibly through amelioration of one or more defects such as the insulin receptor, insulin receptor substrate, glucose transporters, or enzymes involved in the phosphorylation of insulin. The results obtained from the ITT and OGTT tests performed by the same investigators later revealed that the administration of *Ficus carica* reduced the glucose level through the mechanism of insulin-sensitized glucose uptake.

In the presented study, a significant decrease in blood glucose levels were observed in diabetic rats treated with FSO. Sunil *et al.*³⁶⁾ suggested that observed improvement, may be due to the effect of the fig leaves extract either increasing the secretion of insulin from regenerated β -cells or by inhibiting ATP-sensitive K⁺ channels such as glibenclamide, releasing bound insulin from regenerated β -cells.

In diabetes, especially type 2, vascular complications can develop with oxidative stress³⁷⁾. Here, the increase in ROS level may be due to changes in certain enzyme activities such as catalase superoxide dismutase and glutathione peroxidase. Increased antioxidant degradation and/or increased ROS production lead to oxidative stress. Biochemical changes sensitize tissues to oxidative stress, leading to the development of diabetic complications³⁸⁾.

Işler *et al.*³²⁾ were studied on the effect of applying different doses of fig seed oil obtained by cold pressing on experimental myoglobinuric acute kidney injury (MAKI) model. They measured tissue and serum GSH levels in the research groups and GSH levels were significantly decreased compared to FSO groups. The highest score in kidney tissue cast and necrosis levels were observed in the MAKI group, while significant improvements were detected in the treatment groups. They concluded that FSO provided improvement in morphological damage with improvement in functional damage and increase in antioxidative capacity. In our research similar results obtained GSH levels decreased significantly in diabetic rats but increased when FSO added.

Some complications such as stroke, neuropathy, retinopathy and nephropathy have been observed in diabetes due to alterations in many oxidative stress biomarkers such as

lipids, proteins, DNA damage, glutathione, catalase and superoxide dismutase. Oxygen is one of the important components of aerobic life, but under certain circumstances it is highly reactive, harmful and damages molecules and cells by creating reactive species. They cause oxidation through specific mechanisms that interfere with the normal physiological processes of cells. These radicals cause loss of cell function and structure, necrosis and cell death^{39, 40}. An important biomarker for oxidative stress is lipid peroxidation and the resulting malondialdehyde (MDA). Finally, increased production of oxidants by phagocytes, given deficient antioxidant defenses results in chronic oxidative stress and enhances the inflammatory state⁴¹.

Unlike lipids, proteins, which are important building blocks of the body, and their reactions with various oxidants have not been studied in detail. In fact, it has sometimes been assumed that proteins are not particularly susceptible to damage of free radicals. However, this assumption has been invalidated by studies in recent years. As a result of these studies, it has been documented that amino acids, peptides and proteins are also vulnerable to attack from various free radicals and certain oxidants such as lipids⁴¹.

AOPP is an important biomarker in measuring the oxidative modification of proteins. AOPP, formed as a result of the reaction of plasma proteins and chlorinated oxidants such as chloramine and hypochlorous acid (HOCl), functions as the most frequently measured marker of oxidative stress in many diseases⁴². They are most commonly derived from plasma albumins. AOPP should be considered as one of the general biomarkers of oxidative stress, although its molecular compositions have so far been poorly classified^{43, 44}. AOPP levels can often be reversed in various diseases and reference values of healthy individuals are not frequently encountered in the literature. Classical oxidative stress and carbonyl, nitrosative, and chlorine stress have been widely studied in many diseases^{45–47}. Studies in AOPP and diabetes are also very limited. The presented study is also important in this respect.

In addition, the antihyperglycemic effect of FSO was examined histopathologically. This examination is a very important to determine the dextent of the destruction of the pancreas with STZ injection at the microscopic level and how much improvement is achieved when FSO is given in **Table 4**. On histological examination, pancreatic tissues were histologically normal in the control and FSO groups (**Fig. 1**), while severe islet cell degeneration and necrosis in the diabetic group resulted in atrophy of the islets of Langerhans and severe vascular hyperemia (**Fig. 1**). As can be seen from **Table 4**, in the diabetes + FSO group, moderate islet cell degeneration was observed in the pancreatic islet cells and mild hyperemia in the interstitial vessels. A statistically significant difference ($p \leq 0.05$) was found compared to the diabetic group.

In order to determine the intensity of positive staining from the pictures obtained as a result of staining in immunohistochemical and immunofluorescent examinations; statistical test was performed to compare positive immunoreactive cells and immunopositive stained areas with healthy controls. Caspase 3 expression was assessed by immunohistochemistry and was rated as negative in the control and FSO groups. While severe cytoplasmic expression of caspase 3 was detected by immunohistochemistry in the islets of Langerhans in diabetic group, and moderate cytoplasmic expression of caspase 3 in islets of Langerhans cells was demonstrated by immunohistochemistry. A statistically significant difference ($p < 0.05$) was found compared to the diabetic group. Immunohistochemical results are also presented in **Table 5**.

In the study of pancreatic tissues by immunofluorescence, the expression of 8-OHdG was evaluated as negative in the control and FSO groups. In the diabetic group, heavy cytoplasmic expression of 8-OHdG was detected by immunofluorescence in the islets of Langerhans. A slight cytoplasmic expression of 8-OHdG was observed in the islets of Langerhans using immunofluorescence in the Diabetes + FSO group (**Fig. 2**). A statistically significant difference ($p < 0.05$) was found compared with the diabetic group (**Table 5**).

5 Conclusion

Although there are studies on various parts of the fig plant when the literature is examined in detail, there is no published study on the fig seed oil in experimental diabetes yet. It was started with the idea that there are many studies on the hepatoprotective effect of fig fruit, leaf extracts and roots, that there are positive effects, that seed oil can also have similar and positive effects on the pancreas, which is an important organ in metabolism and hormonal effect on glucose carbohydrate regulation. Therefore, the antidiabetic effects of fig seed oil are promising as a result of this study. Documenting that FSO has *in vivo* antioxidant activity, lowers blood sugar in diabetic rats and that STZ-induced pancreatic tissue damage is histopathologically reduced with FSO administration highlights that this oil can be used in human nutrition, pancreatic health, and in diabetics.

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Authors' Contributions

HM has planned the study and the experimental desing. NM and SC did the experimental application. NM performed data analysis and writing of the manuscript. SY did histopathological, immunohistochemical and immunofluorescent examinations.

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