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Histopathological evaluation of the effects of sildenafil on organ damage in a diabetic rat model

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ABSTRACT

Aims: In this study, it was aimed to show the effects of sildenafil on heart, liver and kidney tissues histopathologically by creating an experimental diabetes model with streptozocin.

Methods: Male adult Sprague dawley rat (48) was used in the study. The rats were first divided into three groups as control group, the diabetes group and the diabetes+sildenafil group. Each group was divided into two groups within itself. Streptozotocin 40 mg/kg was administered intraperitoneally to the rats in the groups that would develop diabetes and diabetes+sildenafil diabetes. Rats with blood glucose levels of 250 mg/dl and above after 48 hours were considered diabetic. Sildenafil citrate 10mg/kg/day was given by gavage to the diabetes+sildenafil group. At the end of the experiment heart, liver and kidney tissues were placed in formaldehyde solution. Hematoxylin-Eosin staining was applied to the sections taken. Histological changes in the stained sections were evaluated by a histologist. Histological evaluation was performed semi-quantitatively in heart, liver and kidney tissue. In the assessment, the findings of the tissues were scored and statistical analysis was performed.

Results: Histological findings of heart, liver and kidney tissues were examined. It was determined that less organ damage was seen in the diabetes+sildenafil group compared to the Diabetes group.

Conclusion: In our study, it has been demonstrated histologically that sildenafil can be a drug that has an antioxidant effect in tissue by helping to protect cell structure and architecture against heart, liver and kidney tissue damage caused by diabetes. It should not be overlooked that it is important to determine the appropriate dose and frequency of use of sildenafil in revealing these effects.

Keywords: Sildenafil citrate, diabetes, streptozocin, organ damage

INTRODUCTION

Diabetes is a complex chronic disease in which the number of patients gradually increases with the changing lifestyle, decrease in physical activity and increase in obesity. Reducing the harmful effects of this disease by keeping it under control has been and continues to be the focus of many past studies. Retinopathy, nephropathy, neuropathy, myocardial infarction due to diabetes, and atherosclerosis of the vessels may occur, especially in diabetic patients, due to failure to control hyperglycemia.[1] The underlying problem of these diseases is the increased concentration of free radicals caused by hyperglycemia. Oxidative stress due to free radicals is a known pathogenetic mechanism in diabetic complications.[2] Oxidative/antioxidant balance disrupted by oxidative stress causes macro and microvascular complications of diabetes.[3,4]

Sildenafil, known as a phosphodiesterase type 5 (PDE 5, the enzyme responsible for cGMP hydrolysis) inhibitor, is a therapeutic drug of choice in neurodegenerative diseases,[5] including age-related macular degeneration, used in the treatment of erectile dysfunction and pulmonary arterial hypertension.[6] It is known that sildenafil regulates vascular superoxide release by affecting nitric oxide (NO) release and reduces oxidative stress by causing a vascular antioxidant effect in insulin-resistant rats.[7] In addition, sildenafil is known to regulate oxidative stress markers such as malondialdehyde (MDA),8 reduced glutathione (GSH) and proinflammatory cytokines such as interleukin-4 (IL-4).[9]

These positive effects of sildenafil on oxidative stress are too important to ignore. The multiple effects of diabetes on the organs cause the whole organism to be affected and

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pave the way for the emergence of secondary diseases. The most affected of these organs are the heart, liver and kidney. It will be important to reveal the relationship of sildenafil with diabetes simultaneously through the histological changes in these organs. In this study, it was aimed to show the effects of sildenafil on heart, liver and kidney tissues histopathologically by creating an experimental diabetes model with streptozocin (STZ).

METHODS

The study was carried out with the permission of Erciyes University Animal Experiments Local Ethics Committee (Date: 03.03.2021, Decision No: 21-56). The study adhered to the animal research guidelines of the National Institute of Health.

Experimental Design

48 male adult Sprague Dawley rats weighing 250-300gr were used in the study. The rats were first divided into three groups: The Control group, the Diabetes group, and the Diabetes+Sildenafil group. Each group was divided into two groups within itself (Control 1 group, Control 2 group, Diabetes 1 group, Diabetes 2 group, and Diabetes+Sildenafil 1 group, Diabetes+Sildenafil 2 group). Streptozocin 40 mg/kg was administered intraperitoneally (IP) to the rats in groups Diabetes and Diabetes+Sildenafil that would develop diabetes (dissolved in citrate buffer). Intraperitenoal 1 ml of saline was administered to the control group. Two days after the procedure, blood glucose was measured from the tail vein of the rats, and the rats with a blood glucose level of 250 mg/dl and above were considered diabetic.[10,11] Sildenafil citrate was administered to the Diabetes+Sildenafil group by gavage at 10 mg/kg/day. 1st groups were sacrificed on the 4th day, and the 2nd groups were sacrificed on the 7th day. The reason for dividing the groups within themselves is to show the possible effect of sildenafil as it changes within days. The rats were sacrificed by the decapitation method. The rats were fed as standard in this process and cared for under standard conditions.

Histological Procedure

At the end of the experiment, heart, liver and kidney tissues of rats were fixed in 4% formaldehyde. Then histological tissue follow-up was applied. The tissue was first passed through a graded alcohol series (50%, 70%, 80%, 96%, 3 x Absolute alcohol). Then, it was passed through xylene (x3) and the melt was taken to paraffin. Hematoxylin Eozin (HE) staining was performed to determine histological changes by taking 5 µm sections from the tissues fixed on paraffin blocks. Histological changes in the stained sections were evaluated by a histologist.[12] Histological evaluation was performed semi-quantitatively in heart, liver and kidney tissue. In the assessment, the findings of the tissues were scored and statistical analysis was performed. A total of ten areas in each group were evaluated from tissue sections of each animal, scoring 0: no, 1: slight, 2: moderate, and 3: severe.[13]

Hematoxylin-Eosin Staining

After the preparations of the heart, liver and kidney tissue sections were kept in the oven, the paraffin was removed by taking xylene. Tissues were rehydrated by passing through a series of alcohol (3x Absolute alcohol, 96%, 80%, 70%, 50%). Afterward, the sections were washed with water and kept in a Hematoxylin solution. Sections washed with water again were taken in Eosin solution. The re-washed sections were first examined by passing through the alcohol series, then through xylene and closed with Entellan.[14]

Statistical Analysis

All statistical analyses were carried out by using GraphPad Prism version 7.00 for Mac, GraphPad Software, La Jolla, California, USA. D'Agostino Pearson's omnibus test was used to identify the normal distribution of the data. In the case of normal distribution, quantitative variables were compared using one-way ANOVA analysis of variance and Tukey's posthoc test. The data were expressed as the mean of normalized data standard deviation of the mean. $p<0.05$ was considered statistically significant.

RESULTS

Heart Tissue Histological Findings

Cardiac muscle fibres in the heart tissue of the control 1 group had a normal histological appearance in terms of their regular arrangement, nucleus and cytoplasm. The heart tissue sections of the Control 2 group were also similar to the Control 1 group. Myofibril loss was observed in cardiomyocyte cytoplasm in some areas of the heart tissue belonging to the diabetes 1 group. Disorganization of the cardiomyocyte bundles and loss of myofibrils in the cell cytoplasm were observed in the heart tissue of the diabetes 2 group. Cardiomyocytes, which can be distinguished by their dense eosinophilic cytoplasm, were found in some areas. Cardiomyocyte arrangement in the heart tissue sections of the Diabetes+Sildenafil 1 group was similar to the control groups in terms of cytoplasm and nucleus. In the Diabetes+Sildenafil 2 group, cardiomyocyte bundle disorganization was partially improved compared to the diabetes group. Cardiomyocytes with dense eosinophilic cytoplasm were also less common in this group. Statistical analysis results of heart tissue myofibril loss, cardiomyocyte disorganization and eosinophilic

cardiomyocyte findings are shown in Table 1 and Table 2. The histological findings of all experimental groups are shown in Figure 1.

Table 1. Statistical analysis results of heart tissue myofibril loss, cardiomyocyte disorganization and eosinophilic cardiomyocyte findings in Control 1, Diabetes 1 and Diabetes+Sildenafil 1 groups.

	Control 1	Diabetes 1	Diabetes+ Sildenafil 1	p
Myofibril loss	0.3±0.4[a]	1±0.8[b]	0.5±0.5[ab]	0.0517
Cardiomyocyte disorganization	0.3±0.4	0.8±0.6	0.5±0.5	0.1438
Eosinophilic cardiomyocytes	0.2±0.4[a]	1.1±0.9[b]	0.4±0.5[ab]	0.0181

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Table 2. Statistical analysis results of heart tissue myofibril loss, cardiomyocyte disorganization and eosinophilic cardiomyocyte findings in Control 2, Diabetes 2 and Diabetes+Sildenafil 2 groups.

	Control 2	Diabetes 2	Diabetes+ Sildenafil 2	p
Myofibril loss	0.3±0.4[a]	1.1±0.7[b]	0.6±0.5[ab]	0.0178
Cardiomyocyte disorganization	0.3±0.4	0.8±0.6	0.3±0.4	0.0732
Eosinophilic cardiomyocytes	0.2±0.4[a]	1.1±0.8[b]	0.5±0.5[ab]	0.0127

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Liver Tissue Histological Findings

The liver sections of the Control 1 and Control 2 groups had a regular structure with the vena centralis in the middle forming the classical lobule structure and hepatocyte cell cords and sinusoids radially located towards the periphery. Portal areas located in the periphery of the classical lobule also preserved their regular histological structure. Deterioration was observed in the hepatocyte cords located close to the vena centralis in the classical lobule structure of the liver belonging to the diabetes 1 group. It was observed that some hepatocytes lost their euchromatic nuclei and transformed into cells with denser eosinophilic cytoplasm. In the liver sections of the diabetes 2 group, disruption of radial hepatocyte cords was observed. In addition, cells with altered nuclei and intense eosinophilic staining were observed more frequently in the Diabetes 2 group. While the liver tissue of the Diabetes+Sildenafil 1 group had a generally good appearance, there were almost no cells with an intense eosinophilic appearance. While the sections had a regular appearance in the Diabetes+Sildenafil 2 group, cells with a changed structure and dense eosinophilic cytoplasm were found very rarely. Statistical analysis results of liver tissue hepatocyte cords disorganization and eosinophilic hepatocytes findings are shown in Table 3 and Table 4. The histological findings of all experimental groups are shown in Figure 2.

Table 3. Statistical analysis results of liver tissue hepatocyte cords disorganization and eosinophilic hepatocytes findings in Control 1, Diabetes 1 and Diabetes+Sildenafil 1 groups.

	Control 1	Diabetes 1	Diabetes+ Sildenafil 1	p
Hepatocyte cords disorganization	0.2±0.4	0.7±0.6	0.6±0.6	0.1733
Eosinophilic hepatocytes	0.1±0.3[a]	1.2±0.6b	0.3±0.4[a]	0.0001

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

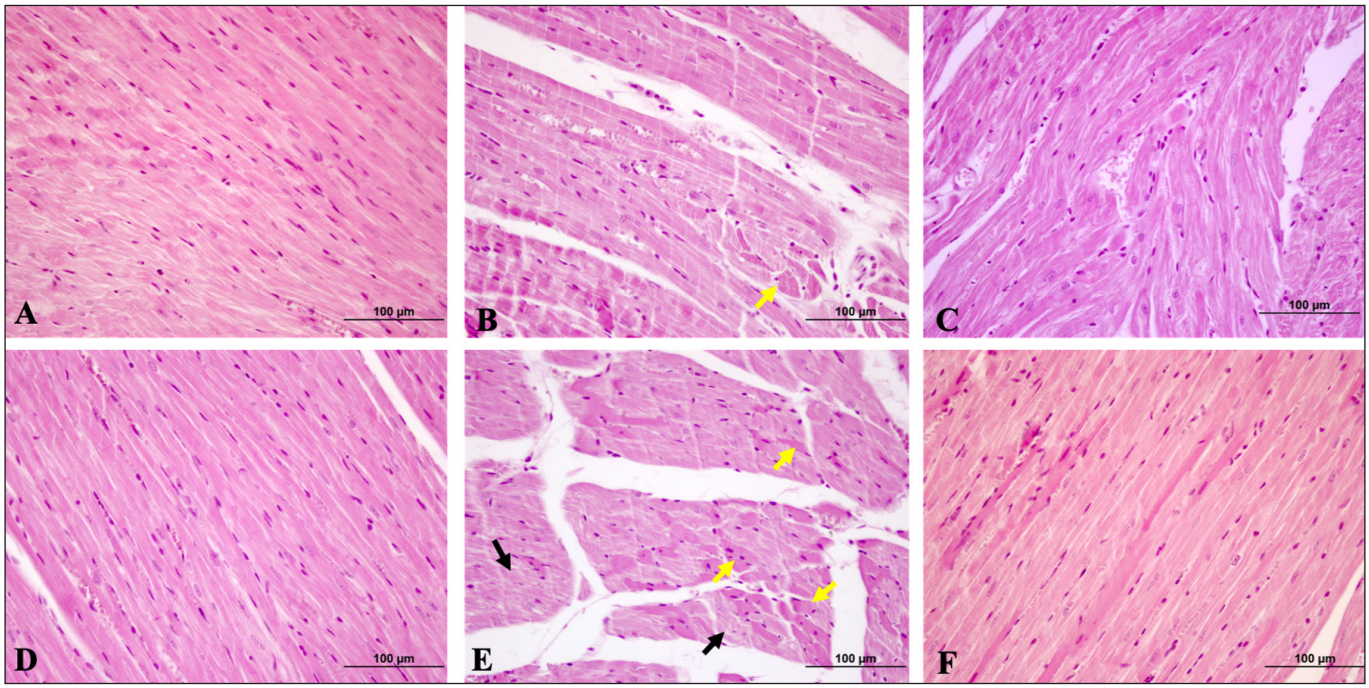

Figure 1. HE staining of heart tissue belonging to the experimental groups. yellow arrow; eosinophilic stained cardiomyocytes, black arrow; shows the loss of myofibrils in cardiomyocytes. A) Control 1 group, B) Diabetes 1 group, C) Diabetes+Sildenafil 1 group, D) Control 2 group, E) Diabetes 2 group, F) Diabetes+Sildenafil 2 group. Scale bar 100 µm.

Table 4. Statistical analysis results of liver tissue hepatocyte cords disorganization and eosinophilic hepatocytes findings in Control 2, Diabetes 2 and Diabetes+Sildenafil 2 groups.

	Control 2	Diabetes 2	Diabetes+ Sildenafil 2	p
Hepatocyte cords disorganization	0.1±0.3[a]	1.2±0.6[b]	0.7±0.4[b]	0.0002
Eosinophilic hepatocytes	0.1±0.3[a]	1.7±0.8[b]	0.2±0.4[a]	0.0001

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Kidney Tissue Histological Findings

In the kidney sections of Control 1 and Control 2 groups, glomeruli, proximal and distal tubule structures forming the nephron, and collecting tubules were evaluated. In both groups, glomerular structure, Bowman's space and parietal leaf were distinguished by their normal appearance. The proximal tubule and distal tubule epithelium had a regular appearance with their cytoplasm and lumens. In general, all cortex and medulla connective tissue and collecting tubules were regular. Histological changes were found mostly in the cortex layer in the diabetes groups. In the diabetes 1 group, disruption of the cytoplasm of the proximal tubule epithelium, scattering in its apical parts, and shed epithelial cells in some tubule lumen was observed. No significant histological changes were observed in the nephron structure and distal tubules. In the diabetes 2 group, epithelial damage in the proximal tubule and cells spilt into the lumen were observed more intensely. Disorganization was also observed in the distal tubule epithelium. In the Diabetes+Sildenafil 1 group, nephron structures had a regular appearance. The number of proximal tubules with epithelial spilt lumen was much lower than in the diabetes groups. Both proximal and distal tubule epithelial cytoplasm also had a more regular appearance. Nephron structures were regular and some disorganization was observed in the proximal tubule epithelium in the Diabetes+Sildenafil 2 group. Shedding was less in some proximal and distal tubule epithelium of the kidney tissue compared to the diabetes group. Statistical analysis results of kidney tissue proximal tubule injury and distal tubule injury findings are shown in Table 5 and Table 6. The histological findings of all experimental groups are shown in Figure 3.

Table 5. Statistical analysis results of kidney tissue proximal tubule injury and distal tubule injury findings in Control 1, Diabetes 1 and Diabetes+Sildenafil 1 groups.

	Control 1	Diabetes 1	Diabetes+ Sildenafil 1	p
Proximal tubule injury	0.2±0.4[a]	1.7±0.6[b]	0.9±0.7[c]	0.0001
Distal tubule injury	0.4±0.5[a]	1.9±0.7[b]	1.1±0.8[ab]	0.0004

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Table 6. Statistical analysis results of kidney tissue proximal tubule injury and distal tubule injury findings in Control 2, Diabetes 2 and Diabetes+Sildenafil 2 groups.

	Control 2	Diabetes 2	Diabetes+ Sildenafil 2	p
Proximal tubule injury	0.4±0.5[a]	1.8±0.7[b]	0.8±0.7[a]	0.0005
Distal tubule injury	0.4±0.5[a]	1.9±0.7[b]	0.9±0.8[a]	0.0003

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

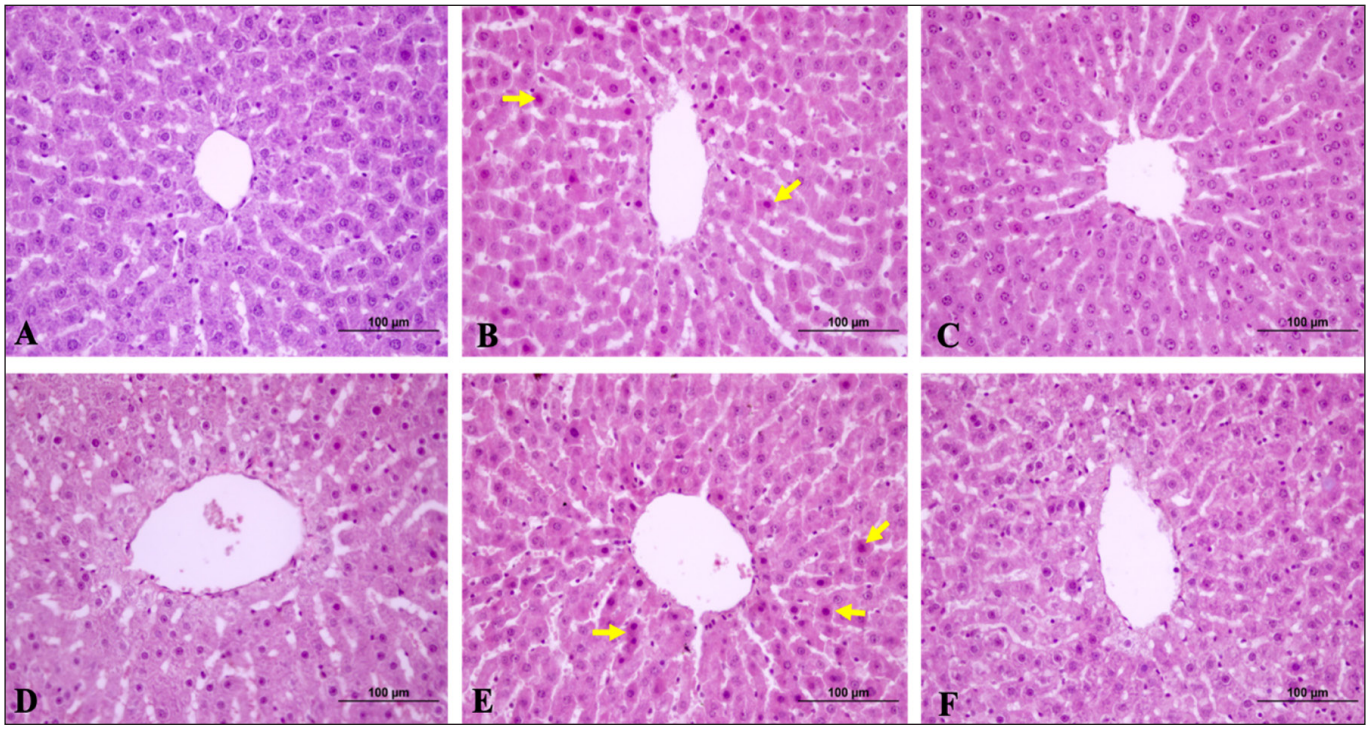

Figure 2. HE staining of liver tissue of the experimental groups. yellow arrow; eosinophilic stained hepatocytes A) Control 1 group, B) Diabetes 1 group, C) Diabetes+Sildenafil 1 group, D) Control 2 group, E) Diabetes 2 group, F) Diabetes+Sildenafil 2 group. Scale bar 100 µm.

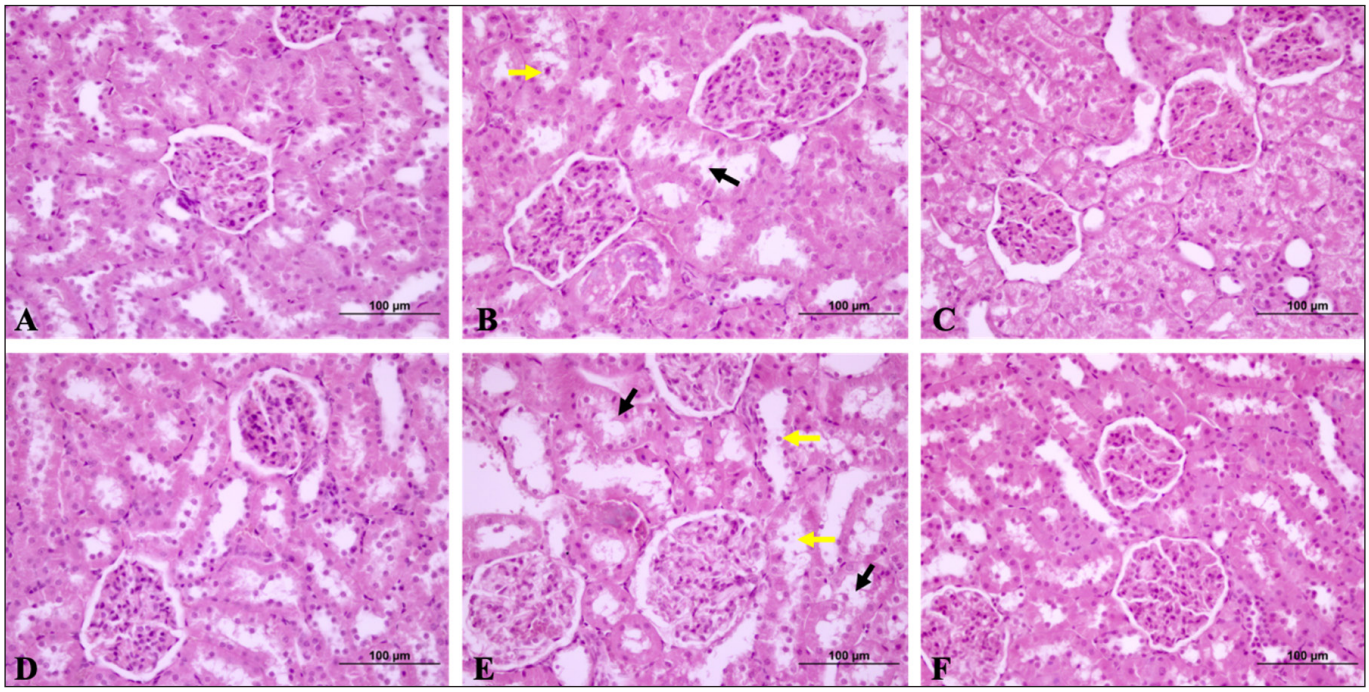

Figure 3. HE staining of kidney tissue of the experimental groups. yellow arrow; epithelial cell spilling into the lumen, black arrow; shows disorganized tubules. A) Control 1 group, B) Diabetes 1 group, C) Diabetes+Sildenafil 1 group, D) Control 2 group, E) Diabetes 2 group, F) Diabetes+Sildenafil 2 group. Scale bar 100 µm.

DISCUSSION

Hyperglycemia from diabetes is a widely recognized cause of increased free radical concentration, whereas the involvement of oxidative stress in glycemic regulation is still debated. Glucose transport is a sequence of events that begins with the interaction of insulin with its receptor on the plasma membrane and ends with intracellular glucose metabolism. Every step plays an important role in this complex sequence of events and can be prevented by reducing oxidative stress.[2] Because elevated blood glucose level stimulates the production of proinflammatory cytokines, and activates lipid peroxidation and apoptotic process, causing various diabetes complications. It has been found that oxidative stress plays a central role in lipid peroxidation, protein oxidation, DNA damage and neuronal death in humans and experimental animals.[15] This study, it was aimed to show the effects of sildenafil on diabetes-induced damage in heart, liver and kidney tissue.

It is known that diabetic rats have a decrease in the activity of antioxidant enzymes and an increase in oxidant enzyme activity, as well as an increase in lipid peroxidation, protein carbonylation, free oxygen radical production, and proinflammatory cytokines. The role of inflammation in the pathogenesis and progression of myocardial damage in chronic diabetes is well established. The detrimental effect of chronic diabetes on the myocardium may result from inflammatory signalling or dysregulation of anti-inflammatory signalling systems.[16] The presence of cytokines due to increased oxidative stress caused by diabetes damages the tissue. In our study, it was concluded that myofibril loss can be prevented and the oxidative and inflammatory response in the tissue can be improved by preserving the cardiomyocyte arrangement, cytoplasm, and nucleus in the heart tissue sections belonging to the Diabetes+Sildenafil group. Considering the properties of sildenafil, it should not be forgotten that its effects on the heart should be revealed not only by histopathological examination but also by methods such as biochemical analyses and gene expression. The appearance of intense eosinophilic cells, especially in the heart and liver tissue, can be considered as an indicator of changes in the cell cytoplasm due to impaired function loss in the tissue. Sildenafil, which is widely used in the treatment of erectile dysfunction, has been reported to reduce the level of IL-8 associated with diabetic cardiomyopathy by suppressing inflammatory processes through cyclic guanine monophosphate (cGMP), which belongs to the drug class that inhibits phosphodiesterase type 5.[17] Sildenafil is known to have strong cardioprotective effects in addition to its antioxidant activity and can reduce apoptosis and necrosis in heart tissues after ischemia-reperfusion injury.[18] Sildenafil also has a relaxing effect on smooth muscle cells of arterioles via cGMP, enhancing its effects through NO production and affecting the liver. NO affects hemodynamic parameters by increasing hepatic microvascular blood flow without increasing pressure due to the relaxation of both presinusoidal stellate cells and portal vein smooth muscle and terminal arterioles, but it is also known that excess NO accumulation is toxic to hepatocytes.[19] It has been reported that liver tissue damage is reduced and hepatocyte cytoarchitecture is regulated

by regulated NO level and antioxidant balance due to sildenafil.[20] In our study, sildenafil was found to be effective in maintaining tissue integrity by regulating the impaired hepatocyte structure and sequence due to diabetes. In the pathogenesis of diabetic nephropathy, it is seen that increased angiotensin II levels cause advanced glycation end products, cytokine increase due to induction of oxidative stress and then kidney tissue damage, especially glomerular damage.[21] Therefore, it can be said that the regulation of NO level in the kidney tissue as in the liver tissue improves the tissue by regulating the abnormal NO and cGMP levels of sildenafil in the damage caused by diabetes. In our study, it was observed that sildenafil was effective in reducing diabetes-related renal tissue tubule damage and protecting the glomerular structure. The absence of biochemical parameters in our study is considered a deficiency, but the histological demonstration that sildenafil produces positive results in tissue in diabetes-induced damage should not be overlooked. In addition to these positive effects, it should be noted that frequent use and high doses of sildenafil may cause damage to liver and kidney tissues.[22]

In our study, a dose of 10 mg/kg/day Sildenafil was applied, and the effect of different doses was not examined. In the studies of Ala et al.[23] it was shown that oxidative stress and antioxidant effects of Sildenafil at different doses (5, 10 and 40 mg/kg/day) may be different, 10 and 40 mg/kg/day doses of Sildenafil were shown to be more effective on oxidative stress and inflammatory cytokines compared to 5 mg/kg/day. Similarly, in the studies of Hafez et al.[24] it was shown that the oxidative stress and antioxidant effects of Sildenafil at different doses (5 and 10 mg/kg/day) may vary. These dose-dependent changes could not be demonstrated in our study because a single dose (10 mg/kg/day) of Sildenafil was administered.

In the study of Jeong et al.[25] it was shown histologically that sildenafil prevented kidney damage in streptozocin-induced diabetic rats. It has been shown histologically that sildenafil prevents kidney damage in models of kidney injury created by different mechanisms. In the study of Jorge et al.[26] it was shown histologically that sildenafil prevented kidney damage in the kidney injury induced by bothrops alternatus snake venom model. These results are similar to our study.

CONCLUSION

In our study, it was revealed that sildenafil may be a drug with antioxidant effects in tissue by helping to preserve cell structure and architecture at the histological level against heart, liver and kidney tissue damage caused by diabetes. It should not be overlooked that determining the appropriate dose and frequency of use of sildenafil is important in revealing these effects.

CONCLUSION

Treatment of ARDS associated with COVID-19 requires a multidisciplinary approach. In this patient group with high mortality and cost, the use of ECMO by considering prognostic factors and guidelines is seen as factors that increase the chance of success. The patients in our study were treated with ECMO in accordance with established guidelines. However, given the high mortality recorded in the present study, we believe that studies on the effectiveness of additional supportive treatments that can reduce ECMO-related complications are needed. As mortality in patients with ARDS due to COVID-19 is higher than that in patients with ARDS unrelated to COVID-19, potential risk factors for mortality other than ARDS need to be reviewed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Animal Experiments Local Ethics Committee (Date: 03.03.2021, Decision No: 21-56).

Informed Consent: This study was designed as an experimental animal study.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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