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# Investigating Oxidative Stress and Histopathological Changes in The Liver of Hyperglycemic Rats

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**ABSTRACT**
**Objective:** This study aimed to investigate the impact of hyperglycemia on the liver in rats. **Materials and Methods:** An experimental diabetes was created by intraperitoneal streptozotocin administration to Sprague-Dawley rats, and blood glucose levels over 250 dl/kg were counted as diabetic. The experiment was continued for 6 weeks, and weight and blood glucose were observed weekly. After sacrification, liver tissues were fixed with formaldehyde and enclosed in paraffin for histological analysis. Hematoxylin and eosin staining was carried out on 4 µm sections taken from paraffin blocks, and the sections were evaluated histopathologically. At the same time, apoptosis was examined immunohistochemically in those sections by active caspase-3 labeling. Finally, oxidative stress status in liver tissue was analyzed by hydrogen peroxide ($H_2O_2$) and superoxide dismutase (SOD) levels. **Results:** The results revealed a significant elevation in H2O2 levels and a slight decline in SOD levels in the liver tissues in the hyperglycemia group. Additionally, apoptotic hepatocyte numbers were elevated. Histopathological analysis revealed disrupted lobular structure, dilatation of the central vein and sinusoids, hepatocyte shrinkage, and lymphocytic infiltration in the hyperglycemia group. **Conclusion:** This study underscores the critical role of apoptosis and oxidative stress in liver dysfunction caused by diabetes, highlighting the need for novel therapeutic strategies to protect against hyperglycemia-induced liver damage and improve outcomes for diabetic patients.
**Keywords:** Experimental Diabetes, Liver, Oxidative Stress, Histopathology, Apoptosis.

# Hiperglisemik Sıçanların Karaciğerindeki Oksidatif Stres ve Histopatolojik Değişikliklerin Araştırılması

**ÖZ**
**Amaç:** Bu çalışmada sıçanlarda hipergliseminin karaciğer üzerine etkisinin değerlendirilmesi amaçlandı. **Gereç ve Yöntem:** Sprague-Dawley tipi sıçanlarda, streptozotosin ile deneysel diyabet modeli oluşturuldu ve kan glikozunun 250 dl/kg'nin üzerinde olduğu sıçanlar diyabetik kabul edildi. 6 hafta süren deney boyunca, haftalık olarak kan glikozu ve ağırlık takibi yapıldı. Sakrifikasyondan sonra alınan karaciğer dokuları, histolojik incelemeler için formaldehit ile fikse edildi ve doku takibinin ardından parafine gömülmüştür. Parafin bloklardan alınan 4 um'lik kesitlerde hematoksilen ve eozin boyaması yapıldı ve kesitler histopatolojik olarak değerlendirildi. Aynı zamanda bu kesitlerde aktif kazpaz-3 işaretlemesi ile apoptoz, immünohistokimyasal olarak incelendi. Son olarak karaciğer dokusundaki oksidatif stres durumu, hidrojen peroksit ($H_2O_2$) ve süperoksit dismutaz (SOD) seviyeleri üzerinden analiz edildi. **Bulgular:** Sonuçlara göre, hiperglisemi grubundaki sıçanlara ait karaciğerlerde $H_2O_2$ düzeyinin anlamlı derecede yükseldiği ve SOD düzeyinin bir miktar düştüğü, hepatositlerde apoptoza giden hücre sayısının arttığı görüldü. Karaciğer histopatolojisi, hiperglisemi grubunda bozulmuş karaciğer lobüler yapısını, santral ven ve sinusoidal dilatasyonları, hepatositlerde küçülmeyi ve lenfositik infiltrasyonu ortaya koydu. **Sonuç:** Bu çalışma, diyabet kaynaklı karaciğer fonksiyon bozukluğunda oksidatif stres ve apoptozun kritik rolünü vurgulayarak, hiperglisemi kaynaklı karaciğer hasarına karşı koruma sağlamak ve diyabetli hastalarda sonuçları iyileştirmek için yeni tedavi stratejilerine olan ihtiyacı vurgulamaktadır.
**Anahtar Kelimeler:** Deneysel Diyabet, Karaciğer, Oksidatif Stres, Histopatoloji, Apoptoz.

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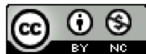

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## INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by persistently high blood glucose levels, arising due to insufficient insulin production or the body's inability to use the insulin it produces effectively. The disease is tried to be kept under control with insulin treatment and lifestyle modification (Crofts, 2015). However, uncontrollable high blood glucose can lead to seconder complications involving the blood vessels, heart, retina, kidneys, and nervous system (Su et al., 2022). Over 400 million people worldwide currently have diabetes, mostly in low- and middle-income countries. By 2045, this number is anticipated to reach 700 million (Saeedi et al., 2019). The destruction of pancreatic β cells by autoimmune mechanisms, which impairs insulin production, characterizes one type of diabetes known as Type 1 diabetes (T1D) (Izadi et al., 2022). Type 2 diabetes (T2D), responsible for over 90% of all cases, involves β-cell dysfunction and insulin resistance (Arte et al., 2024). Both types of diabetes arise from a combination of genetic and environmental factors, and despite ongoing research, no widely effective treatment option has been developed to date. In both types of diabetes, the treatment approach is based on keeping blood glucose balanced, but these approaches are unable to stop the advance of the disease and are insufficient to stop long-term complications (Wu & Mahato, 2014).

Hyperglycemia due to diabetes can damage various tissues and organs in the body if not effectively managed (Burrack et al., 2017). These complications are generally classified as macrovascular diseases, including cardiovascular disease, or microvascular diseases, including retinopathy, nephropathy, and neuropathy (Adu et al., 2019). Diabetes commonly affects the liver, making it one of the organs most impacted by the condition. Studies report a higher prevalence of advanced liver fibrosis in diabetic individuals than non-diabetic individuals (Gao et al., 2024). Additionally, conditions such as hepatitis, cirrhosis, and even hepatocarcinoma are more commonly observed in those with diabetes. A complex relationship also exists between diabetes mellitus and non-alcoholic fatty liver disease (NAFLD), where lipid accumulation in the liver occurs due to hepatic inflammation and oxidative stress (Yang et al., 2019). Although the long-term effects of hyperglycemia on the liver are well documented, the exact mechanisms are still being investigated. Understanding these mechanisms will facilitate the development of supplementary treatments to protect other organs while managing diabetes, for which no definitive cure exists. This is especially important for patients with early-stage diabetes, as such advances could help reduce mortality from diabetes-related complications. Therefore, this study investigated the relationship between liver damage, oxidative stress, and apoptosis in rats exposed to hyperglycemia for six weeks.

## MATERIALS AND METHODS

### Design of the Study

The ethics approval was taken from the Bezmialem Vakıf University Local Ethics Committee (1293-1/ 31.10.2024-E.169810). 12 male Sprague-Dawley rats (12 weeks old) were used and they were maintained on a 12-hour light/dark cycle with unrestricted access to chow and water. The groups were designed as follows (n=6): For the hyperglycemia group (H), experimental diabetes was conducted by intraperitoneal injection of 20 mg/kg STZ (Sigma-Aldrich) in citrate buffer for five days, while controls (C) received only the buffer (Kim et al., 2006). Blood glucose was measured from the tail vein of rats, and above 250 mg/dl were counted as diabetic (Metwally et al., 2018). The experimental process lasted 7 weeks, and the animals were sacrificed. Blood glucose levels were monitored weekly, and body weights were observed throughout the experiment.

### Histological analysis

Small liver tissue samples were fixed in 10% neutral buffered formalin for histological examination. After dehydration in ethanol, the tissues were embedded in paraffin. Paraffin blocks were then sectioned into 4-micrometer slices and stained with hematoxylin-eosin (H&E) (Anapali et al., 2022). Sections were stained with hematoxylin for 20 minutes, rinsed in tap water for 15 minutes, stained with eosin, and processed through a graded alcohol series and toluene. This staining resulted in purple-stained nuclei and pink cytoplasm. The slides were examined for alterations in liver morphology using an Olympus BX53 light microscope. Histomorphometric analyses were performed using the ImageJ program. Sinusoidal expansion and hepatocyte size were calculated as the average of measurements taken from three different regions of a single section per animal (n=5). Group values were expressed in pixels as mean ± SEM.

### Immunohistochemical analysis

The apoptosis was evaluated in liver immunohistochemically. Immunohistochemical analysis was conducted using the following method (Isildar et al., 2022). First of all, the sections were deparaffinized and rehydrated. For the following process, caspase-3 served as the primary antibody (1:500, ThermoFisher, MS-1123), while the secondary antibody provided in the HRP kit was used for detection (Thermo Scientific TP-125-HL). The slides were microwaved in citrate buffer (pH 6.0) for antigen retrieval at two 10-minute intervals. After washing with phosphate-buffered saline (PBS), endogenous peroxidase activity was inhibited by treating the sections with 3% $H_2O_2$ (Merck, Darmstadt, Germany) for 10 minutes. Following another PBS wash, the slides were incubated in the blocking solution for 10 min to minimize non-specific

antibody binding. Primary antibody incubation was performed overnight at +4 °C on the slides. After incubation and washing step, the slides were treated with a biotinylated secondary antibody solution for 10 minutes and rewashed. Streptavidin-peroxidase solution was then applied for 10 minutes, and the reaction was visualized using 3-amino-9-ethyl carbazole (Thermo Scientific TA-125-HA). The samples were examined using light microscopy (Olympus BX53). Analysis was performed by counting active caspase-3 positive cells in 4 separate fields of a single section for each animal and calculating the average per group (n=4).

**Determination of $H_2O_2$ and SOD levels in liver**

For oxidative stress assessment, liver tissues weighing approximately 20 mg were washed in PBS and then homogenized in 180 µL PBS using a tissue lyser at 4°C for 2.5 minutes at 50 oscillations. The homogenates were then centrifuged at 4000 RPM for 10 minutes at 4°C to separate insoluble material. The supernatant was collected and kept on ice for analysis. Protein concentrations in the supernatant were measured using the bicinchoninic acid (BCA) assay (Thermo Scientific, 23225 and 23227). To measure total superoxide dismutase (SOD) activity, 1 mL of working buffer was combined with 0.07 mL of the sample for the test tube, and double-distilled water was used for the control tube. Afterwards, 0.1 mL of nitrosogenic agent, substrate solution, and enzyme solution were added, mixed with a vortex, and incubated at 37°C for 40 minutes. Next, 2 mL of the chromogenic agent was added, mixed, and allowed to incubate at room temperature for 10 minutes. The optical density (OD) was then measured at 550 nm using a quartz cuvette, with double-distilled water as the blank. SOD concentration was calculated based on OD values, and the groups were compared. To measure $H_2O_2$ concentration, 1 mL of buffer solution was added to tubes and incubated at 37°C for 10 minutes. Next, 0.1 mL of double-distilled water, 0.1 mL of 60 mmol/L $H_2O_2$, and 0.1 mL of the sample were added to set up the blank, standard, and sample tubes, respectively. Then, 1 mL of ammonium molybdate reagent was added to each tube and mixed well. The spectrophotometer was calibrated with double-distilled water, and each tube's optical density (OD) was recorded at 405 nm using a quartz cuvette. $H_2O_2$ concentration was calculated based on OD values, and the groups were compared (n=5).

**Statistical analysis**

The statistics were analyzed using SPSS version 20.0. Data are expressed as mean ± standard error. The normality of the data distribution was assessed, and an independent t-test was applied for group comparisons. Statistical significance was set at a p-value of less than 0.05.

**Ethical considerations**

The study received approval from the Bezmialem Vakıf University Local Ethics Committee (Decision No. 1293-1/ 31.10.2024-E.169810, Date: 31.10.2024).

## RESULTS

**Blood glucose levels and body weights of rats**

Following the induction of diabetes with STZ injection, blood glucose levels in the rats began to rise gradually. By the end of the first week, all rats in the hyperglycemia group had glucose levels exceeding 250 mg/dl, meeting the criteria for diabetes. Throughout the study, weekly monitoring showed that glucose levels remained consistently high, over 400 mg/dl. Despite the ongoing hyperglycemia, there were no significant changes in body weight compared to the control group. This indicates that the induction of diabetes did not result in noticeable changes in body weight throughout the experiment.

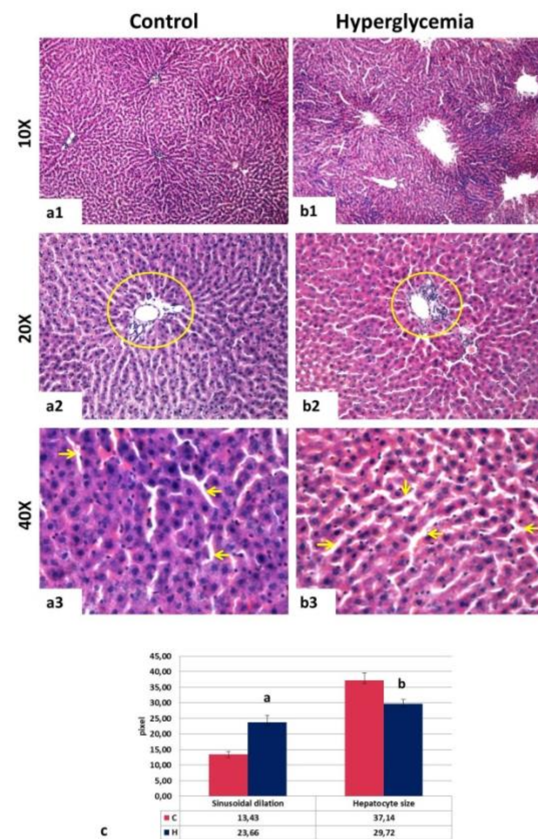


**Figure 1. Representative hematoxylin and eosin stained micrographs of the groups (a-b). C group 10x (a1), 20x (a2), 40x (a3). H group 10x (b1), 20x (b2), 40x (b3). Circle: Portal triad, arrow: sinusoidal spaces. The graphs of sinusoidal dilatations and hepatocyte size (c). $^{a}p < 0.01$, $^{b}p < 0.05$ vs. C groups.**

**Histological analysis**

Histomorphological effects of hyperglycemia on the liver were assessed using H&E stained preparations. Accordingly, the liver sections showed typical lobular structure in the control group, with central veins, portal triad areas, and polygonal hepatocytes separated by sinusoids. Conversely, hyperglycemia group exhibited disrupted lobular organization, enlargement of the central vein, structural changes in

the portal triad areas, lymphocytic infiltration, shrunken hepatocytes, and dilated sinusoidal spaces.

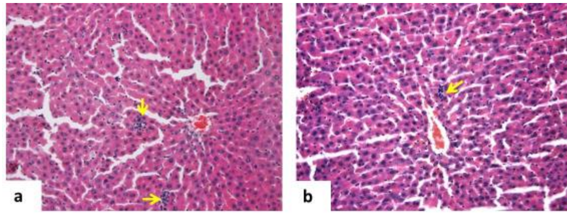


**Figure 2. Representative micrographs of the hyperglycemia group. Arrow: areas of lymphocytic infiltration, 20X, H&E.**

Sinusoidal dilations were seen in the entire liver tissue, and according to the measurements, the average was determined as 13.43±0.2 px in the control group and 23.66±2.27 px in the hyperglycemia group. A statistically significant difference was observed between the groups (p=0.008). The shrinkage of hepatocytes in the hyperglycemia group was also found to be statistically significant. The average hepatocyte size was measured at 37.14±2.39 pixels in the control group, while in the hyperglycemia group, the average size was significantly reduced to 29.72±1.32 pixels (p=0.026). This reduction in hepatocyte size underscores the impact of hyperglycemia on liver cell morphology, further highlighting the pathological changes induced by the diabetic condition. In addition to the reduction in hepatocyte size, cytoplasmic vacuolization and pyknotic nucleus appearance were present in the cells in the hyperglycemia group. Representative micrographs of the groups and the graphs of sinusoidal dilations and hepatocyte size data are given in Fig. 1, and micrographs of lymphocytic infiltration are in Fig.2.

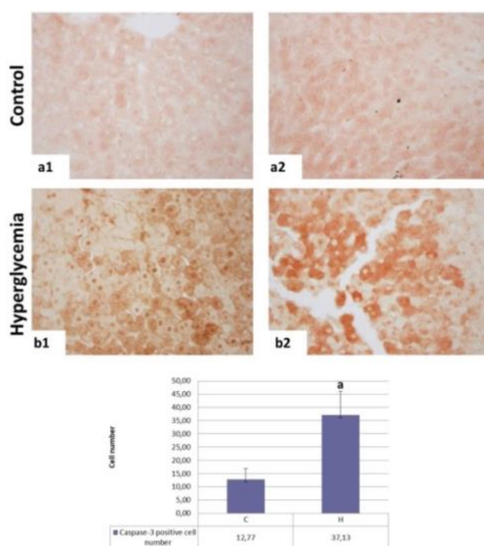


**Figure 3. Representative hematoxylin and eosin stained micrographs of the groups (a-b). The graphs of active caspase-3 positive cell number (c). [a]$p < 0,05$ vs. C group.**

**Immunohistochemical analysis**

Active caspase-3 expression was analyzed immunohistochemically for the evaluation of apoptosis. The mean of active caspase-3-positive cell number revealed that hyperglycemia markedly elevated hepatocyte apoptosis compared to the control group (p=0.047). Representative micrographs of the groups and the data are given in Fig. 3.

**$H_2O_2$ and SOD levels in liver**

In oxidative stress analyses, the $H_2O_2$ level in the liver tissue of normal rats was 0.0401 nmol/g, which significantly increased to 0.0428 nmol/g in the hyperglycemia-induced group (p=0.016). SOD levels, initially 180.7 U/mg in normal rats, decreased to 176.8 U/mg in the hyperglycemia group, but this reduction was not statistically significant. The graph of the results is given in Fig. 4.

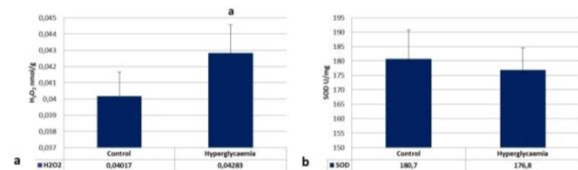


**Figure 4. Graph of $H_2O_2$ concentration in the liver, [a]$p<0.05$ (a). Graph of SOD concentration in the liver (b).**

## DISCUSSION

Diabetes is a metabolic disorder characterized by elevated blood glucose levels. The disease is tried to be kept under control with insulin treatment and lifestyle modification. Secondary complications can arise from poorly controlled high blood glucose, affecting vital organs and systems such as the blood vessels, heart, retina, kidneys, and nervous system. Secondary complications play an important role in diabetes-related deaths (Päth et al., 2019). Identifying the secondary complications caused by diabetes in the body and their underlying mechanisms is essential for developing complementary or supportive treatments to mitigate the effects of uncontrolled hyperglycemia alongside primary diabetes management. Besides, the relationship between diabetes and the liver, which is a vital organ for maintaining homeostasis, has not been studied in the literature as much as the kidney, retina, and nervous system. Here, we demonstrated diabetes-induced liver damage in rats, characterized by histopathological alterations, increased oxidative stress, and enhanced apoptosis.

Histological analysis demonstrated marked alterations in the hepatic lobular structure in the hyperglicemia group. While the control group exhibited typical lobular organization with well-formed central veins, portal triads, and sinusoidal spaces, the hyperglycemia group showed disrupted architecture with enlarged central veins, and dilated sinusoidal spaces. The statistically significant

increase in sinusoidal dilation suggests vascular congestion and altered hepatic blood flow, consistent with previous reports on diabetes-induced microvascular damage (Al-Shaeli et al., 2022; El-Megharbel et al., 2022). Lymphocytic infiltration seen in liver sections of the hyperglycemia group suggests liver damage (Xie et al., 2022). Furthermore, shrunken hepatocytes with cytoplasmic vacuolization and pyknotic nuclei highlight cellular degeneration and stress that may be driven by hyperglycemia and associated metabolic disorders. Considering the increased active caspase-3 expression in the hyerglicemia group, along with the observed histological alterations, it is evident that hyperglycemia induces apoptosis in cells. It has been reported that in long-term exposure to hyperglycemia, hepatocytes undergo senescence and the rate of apoptosis increases (Yuniartha et al., 2022). Hyperglycemia-induced apoptosis has been associated with mitochondrial dysfunction (Hou et al., 2022). Hou et al. applied adipose tissue-derived mesenchymal stem cells (MSCs) as therapeutic agents in an STZ-induced diabetes model and examined their effects in the liver. Accordingly, they demonstrated that adipose tissue-derived MSCs protect the liver from apoptosis by alleviating mitochondrial stress and inflammation (Hou et al., 2022). Since the importance of mitochondrial dysfunction in liver diseases is also known (LeFort et al., 2024), it is obvious that it would be beneficial to analyze mitochondrial function in liver cells.

Oxidative stress analysis further supports the hypothesis of hyperglycemia-induced liver damage. The significant increase in $H_2O_2$ levels has shown the heightened reactive oxygen species (ROS) production, which is a hallmark of diabetic complications (Caturano et al., 2023). Although the change in SOD levels was not statistically significant, this situation indicates that the antioxidant defense system tends to be partially impaired. It is thought that the imbalance caused by increased ROS formation and decreased antioxidant capacity further exacerbates cellular damage, promotes apoptosis, and further impairs liver function. Previous studies have reported increased oxidative stress and reduced antioxidant capacity in the diabetic liver, using various markers to assess these changes and observing oxidative stress dynamics during treatment (Yang et al., 2019; Ye et al., 2023). Considering the findings of our study, it appears that oxidative stress is one of the key parameters in liver damage due to hyperglycemia.

## CONCLUSION

In conclusion, the relationship between oxidative stress and apoptosis observed in this study demonstrates the differential effects of diabetes on the liver. Hyperglycemia-induced ROS production triggers apoptotic signaling pathways, leading to hepatocyte loss, and these changes disrupt hepatic microarchitecture and function, contributing to the pathogenesis of diabetes-associated liver dysfunction, including NAFLD and diabetic hepatopathy. It is crucial to understand how diabetes causes liver damage to develop supplementary treatments that protect against the harmful effects of hyperglycemia and improve the quality of life for diabetic patients. This study highlights the need for new treatment strategies by exploring the molecular pathways that connect hyperglycemia, oxidative stress, and apoptosis, offering a basis for future research.

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The authors declare that there are no acknowledgments.

**Conflict of Interest**
The authors declare that they have no competing interests.

**Author Contributions**
**Plan, design:** BI, MK; **Material, methods and data collection:** BI; **Data analysis and comments:** BI, MK; **Writing and corrections:** BI, MK.

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**Ethical Considerations**
**Institution:** Bezmialem Vakıf University Local Ethics Committee
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## REFERENCES

Adu, M. D., Malabu, U. H., Malau-Aduli, A. E. O., & Malau-Aduli, B. S. (2019). Enablers and barriers to effective diabetes self-management: A multi-national investigation. *PLoS ONE*, *14*(6), e0217771. https://doi.org/10.1371/journal.pone.0217771

Al-Shaeli, S. J. J., Ethaeb, A. M., & Al-Zaidi, E. A. N. (2022). Serological and histological evaluation of the effect of honeybee venom on pancreas and liver in diabetic mice. *Archives of Razi Institute*, *77*(3), 1125–1131. https://doi.org/10.22092/ARI.2022.357385.2025

Anapali, M., Kaya-Dagistanli, F., Akdemir, A. S., Aydemir, D., Ulusu, N. N., Ulutin, T., Uysal, O., Tanriverdi, G., & Ozturk, M. (2022). Combined resveratrol and vitamin D treatment ameliorate inflammation-related liver fibrosis, ER stress, and apoptosis in a high-fructose diet/streptozotocin-induced T2DM model. *Histochemistry and Cell Biology*, *158*(3), 279–296. https://doi.org/10.1007/s00418-022-02131-y

Arte, P. A., Tungare, K., Bhori, M., Jobby, R., & Aich, J. (2024). Treatment of type 2 diabetes mellitus with stem cells and antidiabetic drugs: a dualistic and future-focused approach. In *Human Cell*, *37*(1):54-84. https://doi.org/10.1007/s13577-023-01007-0

Burrack, A. L., Martinov, T., & Fife, B. T. (2017). T cell-mediated beta cell destruction: Autoimmunity and alloimmunity in the context of type 1 diabetes. In *Frontiers in Endocrinology*, 8, 343. https://doi.org/10.3389/fendo.2017.00343

Caturano, A., D'Angelo, M., Mormone, A., Russo, V., Mollica, M. P., Salvatore, T., Galiero, R., Rinaldi, L., Vetrano, E., Marfella, R., Monda, M., Giordano, A., & Sasso, F. C. (2023). Oxidative stress in type 2 diabetes: Impacts from pathogenesis to lifestyle modifications. In *Current Issues in Molecular Biology*, *45*(8), 6651–6666. https://doi.org/10.3390/cimb45080420

Crofts, C. A. P. (2015). Hyperinsulinemia: A unifying theory of chronic disease? *Diabesity*, 6, 35. https://doi.org/10.15562/diabesity.2015.19

El-Megharbel, S. M., Al-Baqami, N. M., Al-Thubaiti, E. H., Qahl, S. H., Albogami, B., & Hamza, R. Z. (2022). Antidiabetic drug sitagliptin with divalent transition metals manganese and cobalt: Synthesis, structure, characterization antibacterial and antioxidative effects in liver tissues. *Current Issues in Molecular Biology*, *44*(5), 1810–1827. https://doi.org/10.3390/cimb44050124

Gao, L., Wang, X., Guo, L., Zhang, W., Wang, G., Han, S., & Zhang, Y. (2024). Sex differences in diabetes-induced hepatic and renal damage. *Experimental and Therapeutic Medicine*, *27*(4), 148. https://doi.org/10.3892/etm.2024.12436

Hou, Y., Ding, W., Wu, P., Liu, C., Ding, L., Liu, J., & Wang, X. (2022). Adipose-derived stem cells alleviate liver injury induced by type 1 diabetes mellitus by inhibiting mitochondrial stress and attenuating inflammation. *Stem Cell Research and Therapy*, *13*(1), 132. https://doi.org/10.1186/s13287-022-02760-z

Isildar, B., Ozkan, S., Ercin, M., Oktayoglu, S. G., Oncul, M., & Koyuturk, M. (2022). 2D and 3D cultured human umbilical cord - derived mesenchymal stem cell - conditioned medium has a dual effect in type 1 diabetes model in rats : immunomodulation and beta - cell regeneration. *Inflammation and Regeneration*, *42*(1), 55. https://doi.org/10.1186/s41232-022-00241-7

Izadi, M., Sadr Hashemi Nejad, A., Moazenchi, M., Masoumi, S., Rabbani, A., Kompani, F., Hedayati Asl, A. A., Abbasi Kakroodi, F., Jaroughi, N., Mohseni Meybodi, M. A., Setoodeh, A., Abbasi, F., Hosseini, S. E., Moeini Nia, F., Salman Yazdi, R., Navabi, R., Hajizadeh-Saffar, E., & Baharvand, H. (2022). Mesenchymal stem cell transplantation in newly diagnosed type-1 diabetes patients: a phase I/II randomized placebo-controlled clinical trial. *Stem Cell Research & Therapy*, *13*(1), 264. https://doi.org/10.1186/s13287-022-02941-w

Kim, E., Sohn, S., Lee, M., Jung, J., Kineman, R. D., & Park, S. (2006). Differential responses of the growth hormone axis in two rat models of streptozotocin-induced insulinopenic diabetes. *Journal of Endocrinology*, *188*(2), 263–270. https://doi.org/10.1677/joe.1.06501

LeFort, K. R., Rungratanawanich, W., & Song, B. J. (2024). Contributing roles of mitochondrial dysfunction and hepatocyte apoptosis in liver diseases through oxidative stress, post-translational modifications, inflammation, and intestinal barrier dysfunction. In *Cellular and Molecular Life Sciences*, *81*(1), 34. https://doi.org/10.1007/s00018-023-05061-7

Metwally, M. M. M., Ebraheim, L. L. M., & Galal, A. A. A. (2018). Potential therapeutic role of melatonin on STZ-induced diabetic central neuropathy: A biochemical, histopathological, immunohistochemical and ultrastructural study. *Acta Histochemica*, *120*(8), 828–836. https://doi.org/10.1016/j.acthis.2018.09.008

Päth, G., Perakakis, N., Mantzoros, C. S., & Seufert, J. (2019). Stem cells in the treatment of diabetes mellitus — Focus on mesenchymal stem cells. *Metabolism: Clinical and Experimental*, *90*, 1–15. https://doi.org/10.1016/j.metabol.2018.10.005

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., & Williams, R. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, 157, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Su, W., Yu, S., Yin, Y., Li, B., Xue, J., Wang, J., Gu, Y., Zhang, H., Lyu, Z., Mu, Y., & Cheng, Y. (2022). Diabetic microenvironment preconditioning of adipose tissue-derived mesenchymal stem cells enhances their anti-diabetic, anti-long-term complications, and anti-inflammatory effects in type 2 diabetic rats. *Stem Cell Research and Therapy*, *13*(1), 422. https://doi.org/10.1186/s13287-022-03114-5

Wu, H., & Mahato, R. I. (2014). Mesenchymal stem cell-based therapy for type 1 diabetes. *Discovery Medicine*, *17*(93), 139–143.

Xie, Y., Zhong, K. B., Hu, Y., Xi, Y. L., Guan, S. X., Xu, M., Lin, Y., Liu, F. Y., Zhou, W. J., & Gao, Y. (2022). Liver infiltration of multiple immune cells during the process of acute liver injury and repair. *World Journal of Gastroenterology*, *28*(46), 6537–6550. https://doi.org/10.3748/wjg.v28.i46.6537

Yang, H., Yang, T., Heng, C., Zhou, Y., Jiang, Z., Qian, X., Du, L., Mao, S., Yin, X., & Lu, Q. (2019). Quercetin improves nonalcoholic fatty liver by ameliorating inflammation, oxidative stress, and lipid metabolism in db/db mice. *Phytotherapy Research*, *3*(12), 3140–3152. https://doi.org/10.1002/ptr.6486

Ye, H., Sun, M., Jin, Z., Yuan, Y., & Weng, H. (2023). FTY-720 alleviates diabetes-induced liver injury by inhibiting oxidative stress and inflammation. *Fundamental and Clinical Pharmacology*, *37*(5), 960–970. https://doi.org/10.1111/fcp.12897

Yuniartha, R., Arfian, N., Setyaningsih, W. A. W., Kencana, S. M. S., & Sari, D. C. R. (2022). Accelerated senescence and apoptosis in the rat liver during the progression of diabetic complications. *Malaysian Journal of Medical Sciences*, *29*(6), 46–59. https://doi.org/10.21315/mjms2022.29.6.5