CUKUROVA MEDICAL JOURNAL

ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cilt/Volume 38, Sayı/Number 1, Yıl/Year 2013

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Cukurova Medical Journal

ISSN: 0250-8150 (Print)
Periodical of Cukurova University Faculty of Medicine
Journal Home Page

Language: [Turkish] [English]

- 2016
  - Volume 41, Issue 3 pp. 423-611
  - Volume 41, Issue 2 pp. 201-421
  - Volume 41, Issue 1 pp. 1-199
+ 2015
+ 2014
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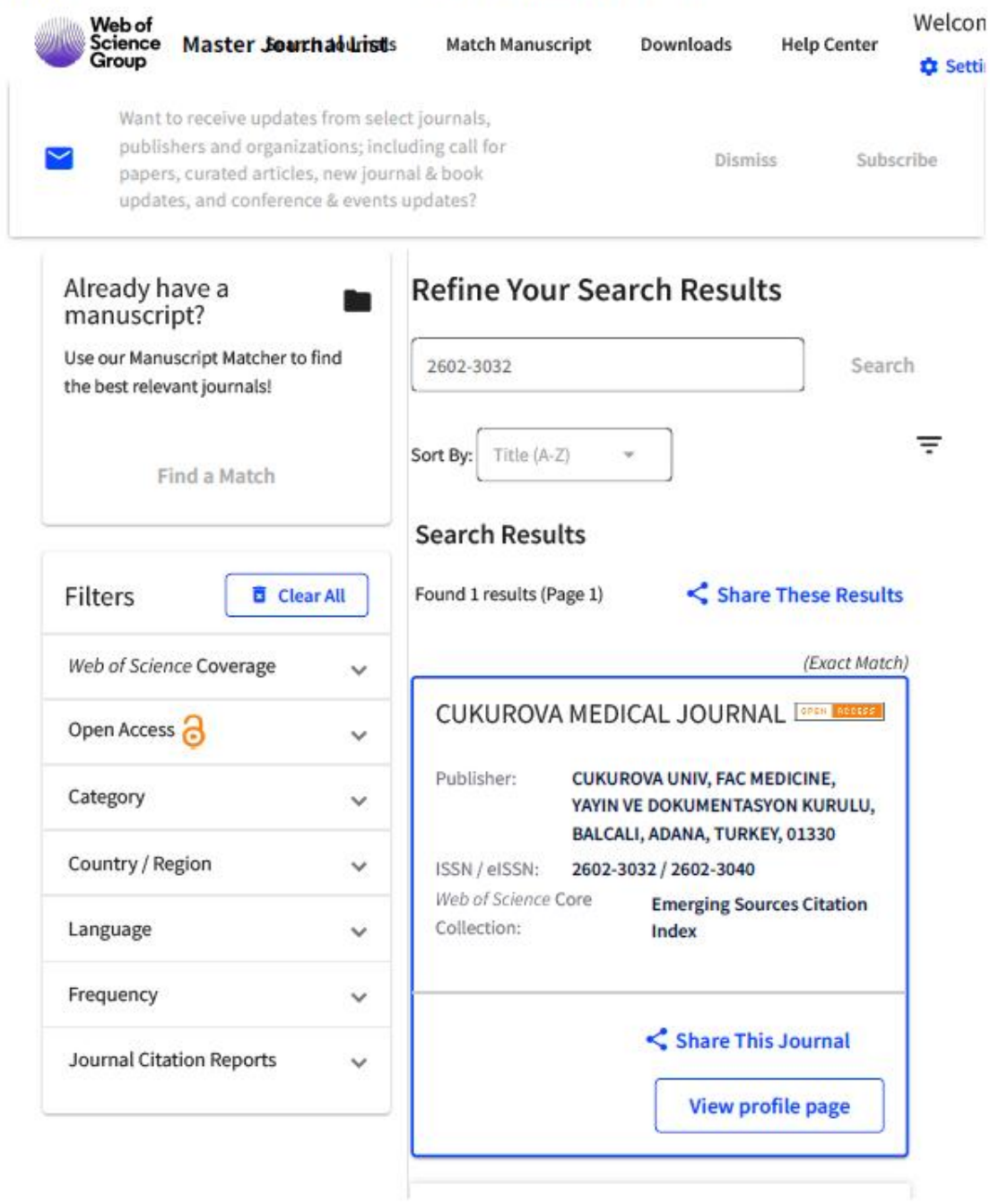
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Cukurova Med J 2020;45(4):1733-1741
DOI: 10.17826/cumj.791369

ARAŞTIRMA / RESEARCH

Oxidative stress markers in liver in streptozocin-induced diabetic rats: effects of metformin and sitagliptin

Streptozotosin kaynaklı diyabetik sıçanların karaciğerindeki oksidatif stres belirteçleri: metformin ve sitagliptinin etkileri

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Cukurova Medical Journal 2021;46(1):1733-1741

Abstract

Purpose: This study aims at investigating the oxidative stress effect of antidiabetic drugs of Metformin (MET) and sitagliptin (STG) in the liver tissue of diabetic rats from streptozotocin (STZ).

Materials and Methods: Thirty-five female Wistar rats (3-4 months old, weighing 200±25 g) were divided into five groups (with seven rats each) and treated as follows: control (Cont), streptozotocin alone (STZ), streptozotocin + metformin (STZ+MET), streptozotocin + sitagliptin (STZ+STG), streptozotocin + metformin + sitagliptin (STZ+MET+STG). Sitagliptin, Metformin, and combined metformin sitagliptin treated subgroups for four weeks. Malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS) levels, and oxidative stress index (OSI) ratio were measured in rat liver tissue. Besides, cells undergoing apoptotic cell death were determined using the TUNEL technique through histopathological evaluation.

Results: MDA, TAS, and OSI of STZ+MET+STG administered group decreased compared to STZ. TOS of STZ+MET+STG administered group decreased compared to STZ.

Conclusion: In the experimental T2D model in rats, it shows protective effect when sitagliptin is used with metformin against oxidative damage in liver tissue caused by STZ.

Keywords:. Metformin, sitagliptin, oxidative stress, diabetes mellitus

Öz

Amaç: Bu çalışmanın amacı, streptozotosin (STZ) kaynaklı diyabetik sıçanların karaciğer dokusunda metformin (MET) ve/veya sitagliptinin (STG) antidiyabetik ilaçların olası oksidatif stres etkisini araştırmaktır.

Gereç ve Yöntem: Bu çalışmada erişkin dişi Wistar albino sıçanları kullanılmış ve rastgele kontrol normal grubu, kontrol diyabetik grubu ve diyabetik gruba ayrılmıştır. Antidiyabetik ilaç grubuna sahip diyabetik sıçanlar aşağıdaki tedavi edilen alt gruplara ayrıldı: Sitagliptin (10 mg/ kg/ gün), Metformin (200mg/ kg/ gün) ve Kombine metformin sitagliptin ile 4 hafta boyunca tedavi edilen alt gruplar. Sıçan karaciğer dokusunda oksidatif stres belirteçleri (Malondialdehid (MDA), total antioksidan durum (TAS), total oksidan durum (TOS) ve oksidatif stres indeksi (OSI) seviyeleri) ölçüldü. Ek olarak, apoptotik hücre ölümü geçiren hücreler histopatolojik değerlendirme ile *TUNEL* tekniği kullanılarak belirlendi.

Bulgular: STZ + MET + STG uygulanan grubun MDA, TAS ve OSI'si STZ'ye göre azaldı. STZ + MET + STG uygulanan grubun TOS'u STZ'ye göre azaldı.

Sonuç: Sıçanlarda deneysel T2D modelinde, sitagliptin metformin ile birlikte kullanıldığında karaciğer dokusunda STZ'nin neden olduğu oksidatif hasara karşı koruyucu etki göstermektedir.

Anahtar kelimeler: Metformin, sitagliptin, oksidatif stres, diyabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is an important problem of public health with growing incidence and prevalence worldwide. This chronic disease can affect multiple vital organs such as the liver, kidney, heart, brain[1-4]. It has been reported that excessive fat accumulation in the liver adversely affects insulin resistance leading to metabolic dysfunction[5]. Insulin stores glucose and inhibits glucagon secretion. It also causes a decrease

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Geliş tarihi/Received: 07.09.2020 Kabul tarihi/Accepted: 11.10.2020 Çevrimiçi yayın/Published online: 30.12.2020

in liver glucose production by lowering the concentration of serum fatty acids. This situation can result in lipid peroxidation in muscle cell membranes, contributing to the formation of insulin resistance[6].

Both exogenous and endogenous substances generate free radicals in cells. These free radicals formed cause oxidation of cell components and molecules and eventually damaged the cell[7]. There are antioxidant systems that include enzymatic and non-enzymatic antioxidants, which are generally effective in preventing the detrimental effects of free radicals[8]. Oxidative stress associated with many diseases, including neurological disorders, atherosclerosis, diabetes, cancer, and hypertension means a change of the balance antioxidants and oxidants favoring oxidants[6,8].

Metformin, a *biguanide* antidiabetic medication, is used for T2D. The effect of metformin is manifested as a decrease in hepatic glucose level and an increase in peripheral glucose excretion, responding to increased insulin in the liver[9]. Acording to several studies metformin abates endogenous ROS (including free radicals) mitochondrial levels[10-13]. Sitagliptin (STG) is a dipeptidyl peptidase-4 (DPP 4) inhibitor**.** Type 2 diabetes can be treated with an oral antidiabetic agent called sitagliptin. It was reported that the DPP-4 inhibitors have effects on lipid profile, oxidative stress, hepatosteatosis, reducing inflamation and insulin resistance. Sitagliptin suppresses glucagon secretion due to DDP-4 inhibition, thus stimulating the liver and decreasing glucose production. In some studies in recent years, STG has been reported to show antioxidative effects[14-17]. This study investigates the oxidative stress effects of metformin and sitagliptin in the liver in a type 2 diabetes mellitus rat model.

MATERIAL AND METHODS

Animals and treatment design

This study was conducted in Çanakkale Onsekiz Mart University Experimental Research Center (ÇOMÜDAM). Five study groups were formed using 35 adult female Wistar albino rats with free access to water and food. All rats were housed in metabolic cages on a natural daily 12 h light/dark cycle at 25-27 °C. All experiments were conducted under the Guide for the Care and Use of Laboratory Animals . Following overnight fasting, a single intraperitoneal injection of STZ (40 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) induced experimental diabetes. Fasting blood glucose (FBG) levels were tested three days after STZ injection, and the rats which had high FBG (250 mg/dL) were used for the subsequent experiments [19].

Inclusion criteria: Weight between 170 g and 230 g, female rats, survival after treatment. Exclusion criteria: The weight less than 150 g or greater than 280 g, male rats, the dead rats after treatment.

There was the equal classification of diabetic rats into the following four groups as follows (n=7):

Group 1: control (Cont);
Group 2: streptozotocin (STZ)
Group 3: streptozotocin + metformin (STZ+MET);
Group 4: streptozotocin + sitagliptin (STZ+STG);
Group 5: streptozotocin + metformin + sitagliptin (STZ+MET+STG).

In the study groups, metformin (*Metformin*, *Glukofen*, Ilsan-Hexal, İstanbul) (200 mg/kg/day) and sitagliptin (Januvia, Merck Sharp & Dohme İlaçları Ltd. Şti., Turkey) (10 mg/kg/day) were treated via oral gavage. The follow-up period was four weeks in all groups. The ethical approval for the use of rats was obtained from the Local Ethics Board for Experimental Animals of Çanakkale Onsekiz Mart University (COMU-HADYEK/2020-04-04). This study was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health[18].

Tissue preparation

Once this follow-up period ended, a mixture of ketamine and xylazine (75 mg/kg and 10 mg/kg, respectively) was used to anesthetize and sacrifice all animals. Rats' livers were rapidly dissected, and weighed. After removing of liver tissues, 0.1 M phosphate buffer, and EDTA, pH = 7.4 (1:10) were used to homogenize the tissues. Centrifugation of the prepared liver homogenate was done at 7,000 × g for 5 min.

Measurements

Malondialdehyde (MDA)

The level of tissue MDA was determined spectrophotometrically based upon heating the sample under acidic conditions. Thiobarbituric acid (TBA) is reacted with MDA, resulting in the production of pink pigment. Briefly, the mixture was heated in a boiling water bath at 90 °C for 15 min.

(The absorbance was read at 532 nm after cooling. The results were expressed as nmol/g wet tissue (Relassay, Turkey).

Total oxidant status (TOS)

Kits commercially available (Relassay, Turkey) were used to measure TOS levels. The ferrous ion-o-dianisidine complexes containing iron are oxidized to ferric ions by the oxidants in the sample. The glycerol molecules in the reaction medium enhancer this oxidation reaction. The color intensity is measured spectrophotometrically, in which a colored complex with xylenol orange in an acidic medium, is formed by the ferric ions and related to the total oxidant molecules in the sample. The results were expressed as micromolar hydrogen peroxide equivalent per liter (μmol H2O2 equivalent/L) [20].

Total antioxidant status (TAS)

Levels of TAS were measured using commercially available kits (Relassay, Turkey). This method is based on the decolorized of the ABTS (2,2 ' - Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. This situation is related to its antioxidant capacity. The results were expressed as mmol Trolox equivalent/L [21].

Oxidative stress index (OSI)

The oxidative stress index (OSI) means the TOS level to the TAS level ratio. Briefly, the OSI formula is: OSI (arbitrary unit) = TOS (μmol H2O2 equivalent/L) / TAS (μmol Trolox equivalent/L) [22-24].

Histological procedures

Once the experiment, was ended, anesthetized rats' liver tissues were taken and trimmed, then placed in tissue transport cassettes and fixed in immunofix for 24 hours. Afterward, tissue samples are passed through graded alcohol solutions and purified from the water in the tissue. The alcohol in the tissues was cleaned with xylene, the tissue samples were kept in the paraffin in the oven and then blocked in the base mode, the blocked tissue samples were cut with a microtome at a thickness of 3-5 microns and placed in preparation boxes. Tissue samples taken from each subject and cut at a thickness of 5 microns were obtained by H.E. painting was applied[25].

TUNNEL assay

The Terminal Transferase dUTP Nick End Labeling (TUNEL, S7101, Millipore, USA) method, which allows staining of apoptotic cells, was used to determine cell death. First, distilled water and then PBS solution was used to wash the sction for 3x5 minutes following the deparaffinization process. Then, 20-μg / ml Proteinase-K diluted 1/500 with PBS solution was applied at room temperature for 15 minutes. After washing with PBS, it was treated with 3% H_2O_2 for 5 minutes, and then washed 3x5 minutes again with PBS. The samples were kept at room temperature for 5 minutes with Equilibration buffer, and then kept for 1 hour at 37 ^oC in a humid environment with TdT-enzyme. After the period, samples treated with Stop Wash Buffer for 10 minutes and then with Antidioxygenin Peroxidase. Conjugate for 30 minutes were washed 3x5 minutes with PBS. It was then stained with DAB, then washed with distilled water. Background painting was done with Mayer's Hematoxylin.

Apoptotic index; TUNEL stained cell number and percentages of apoptosing cells were determined by quantitating the percentage of labeled nuclei by light microscope, scoring not less than 1,000 adjacent cells [26].

Statistical analysis

Results are presented as mean ± SD. SPSS, version 19.0. (SPSS, IBM Company) was used to analyze to the differences between the groups for performance, biochemical and histological parameters. The data distribution normality was examined with the Kolmogorov-Smirnov normality test. In comparing the patients and controls, Mann-Whitney U and Kruskal-Wallis tests were used for continuous variables, and a chi-square test was used for categorical variables. The paired comparison of the data was performed with Duncan's test. P values below 0.05 were regarded as significant.

RESULTS

Results and comparisons of liver tissue MDA, TAS, TOS, and OSI levels of the five groups were given in Table 1. MDA levels of the STZ group elevated compared to those of controls (p=0.001). MDA levels of STZ+MET, STZ+STG, and STZ+MET+STG administered groups decreased compared to those of STZ (p=0.001). Liver TAS levels of the STZ administered group decreased compared to controls (p=0.001). TAS levels of STZ+MET administered group increased compared to STZ (p=0.01), while TAS levels of STZ+MET+SIT administered group recovered

(p=0.001). TOS levels of STZ administered groups elevated compared to those of controls (p= 0.001). TOS levels of STZ+MET STZ+STG and STZ+MET+STG administered group decreased compared to STZ (p=0.001), and TOS levels of STZ+MET+STG administered group recovered (p=0.001).

STZ administered group had higher OSI than controls (p=0.001). OSI of STZ+MET STZ+STG and STZ+MET+STG administered group lower compared to STZ (p=0.001), and OSI of STZ+MET+STG administered group recovered (p=0.001).

Table 1. Spectrophotometric analysis results.

Group	MDA (nmol/g)	TAS (mmol Trolox Equiv/L)	TOS (μmol H2O2 Equiv/L)	OSI (Arbitrary Unit)
1 Control	0.93±0.02	2.39±0.01	23.89±0.07	1.35±0.01
2 STZ	1.10±0.01 A*	1.77±0.02 A*	36.10±0.07 A*	1.51±0.01 A*
3 STZ+MET	0.79±0.05 B*, E*	1.86±0.04 B*, E*	29.60±2.58 B*, E*	1.59±0.16 B*, E*
4 STZ+STG	0.85±0.11 F*	1.80±0.08 C*	29.75±2.90 C*, F*	1.65±0.10 C*,F*
5 STZ+MET+STG	0.66±0.01 D*, G*	2.25±0.02 D*, G*	26.33±0.05 D*, G*	1.17±0.01 D*, G*

Data are given as mean±SD. * p< 0.05. Group Comparisons: A: 1 and 2, B: 1 and 3, C: 1 and 4, D: 1 and 5, E: 2 and 3, F: 2 and 4, G: 2 and 5. Abbreviations: STZ, Streptozotocin; MET, Metformin; STG, Sitagliptin; MDA, Malondialdehyde; TAS, Total Antioxidant Status, TOS, Total Oxidant Status; OSI, Oxidative Stress Index

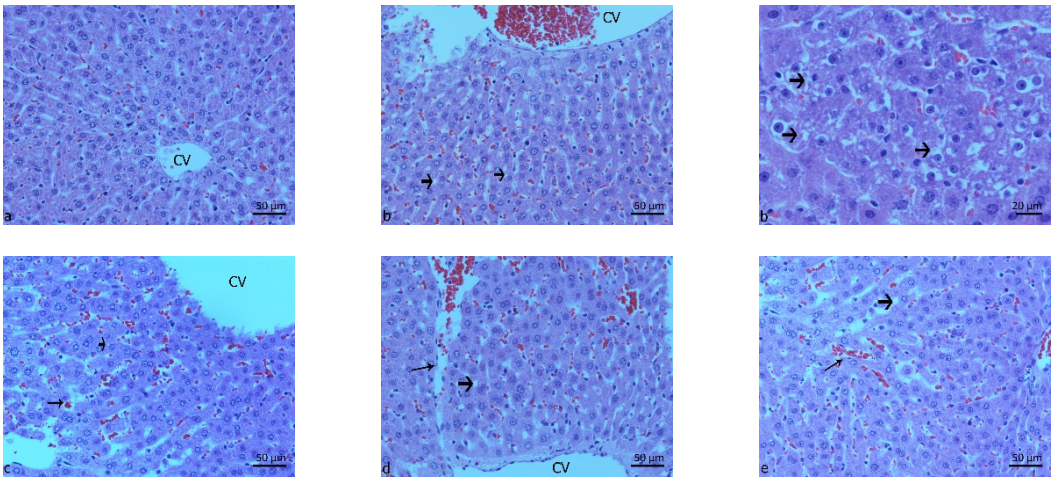


Figure 1. The histopathological picture in rat groups a) Control group liver tissue (CV: Central vein), b) Liver tissue of the second group of diabetic control (arrow: vacuolar degeneration), c) Liver tissue of the third group of MET treatment (long arrow: sinusoidal dilatation, short arrow: vacuolar degeneration), d) Liver tissue of the fourth group of STG treatment (long arrow: sinusoidal dilatation, short arrow: vacuolar degeneration), e) Liver tissue of the fifth group of MET+STG combined treatment (long arrow: sinusoidal dilatation, short arrow: vacuolar degeneration) Scale bar: 50 µm.

In the histological staining performed with routine Hematoxylin-Eosin, no histopathological picture was found in rats' the liver tissue in the control group. The trailer structure formed by hepatocytes and the

lobular structure of the tissue were normal. In DM group rats, the most prominent findings were diffuse degeneration and hepatocytes' necrosis. It was found that increased oxidative stress due to high glucose levels in the liver tissue of the STZ group caused nonalcoholic fatty liver, hepatocyte vacuolization, and sinusoidal dilatation. It was also observed that inflammatory cell infiltration increased in portal areas. It has been determined that there is little mitotic activity. As a result of treatment with metformin and sitagliptin, these findings were found to be significantly reduced. While vacuolations were formed in the cytoplasm of hepatocytes in the STZ + MET and STZ + STG groups, which are among the treatment groups, it was determined that the histopathological picture decreased significantly in the STZ + MET + STG group and had a histological appearance similar to the control group (Figure 1).

While the programmed cell death mechanism mostly occurs in embryonic tissues during the prenatal period, disruption of oxidant-antioxidant balance and tissue damage in adult tissue are important factors that trigger apoptosis. In hepatocellular damage, especially oxidative stress and inflammation cause hepatocytes to drift into apoptosis by cytokines released from Kupffer cells. Failure of functional processes in the tissue is one of the important triggering conditions for apoptosis. In the experimental diabetes model, it has been observed that apoptosis due to oxidative stress is triggered and the cells undergoing apoptosis increase in the tissue. While the apoptotic process was at the highest level in the STZ applied group, the least apoptotic cell was found in the liver tissues of the STZ + MET + SGT applied groups compared to the other groups. It was observed that there were fewer STZ + MET + SGT cells in the liver tissues of the STZ + MET active substance groups and more apoptotic cells than the STZ group. Among the substances applied to the experimental groups, it was determined that the treatment method with the most protective properties of hepatocytes from apoptosis due to high glucose levels of diabetes was provided with the combination of MET + SGT. The highest significance between TUNEL positivity groups was determined as *** $p<0.001$ between STZ and control groups. The least significance was observed between STZ + MET and STZ + SGT groups (*$p<0.05$), while it was observed as ** $p<0.01$ between STZ + MET + SGT and STZ + MET groups. As a result, the apoptotic index value was high in the STZ group and low in the STZ + MET + SGT group (Figure 2, Figure 3).

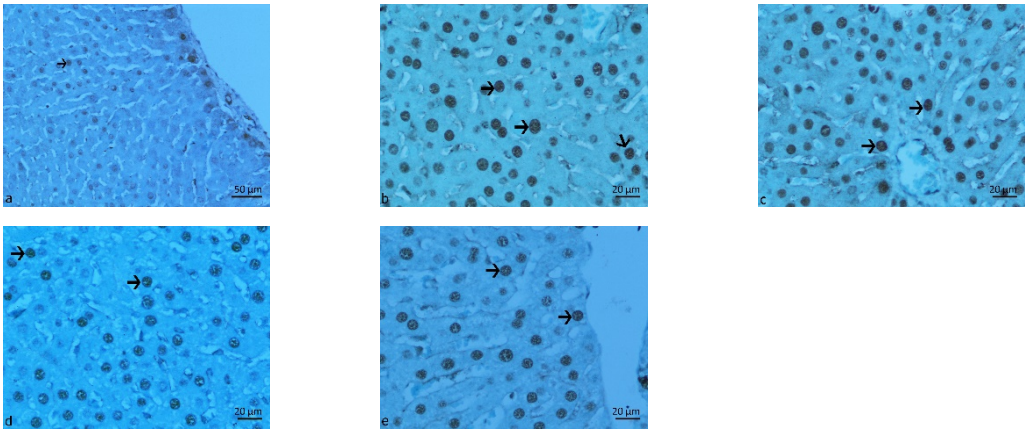


Figure 2. The TUNEL assay in the liver tissue (Arrow: apoptotic cells, a: control group, b: diabetic control, c: STZ+MET treatment, d: STZ+STG treatment, e: STZ+MET+STG treatment) Scale bar: 50 µm and 20 µm.

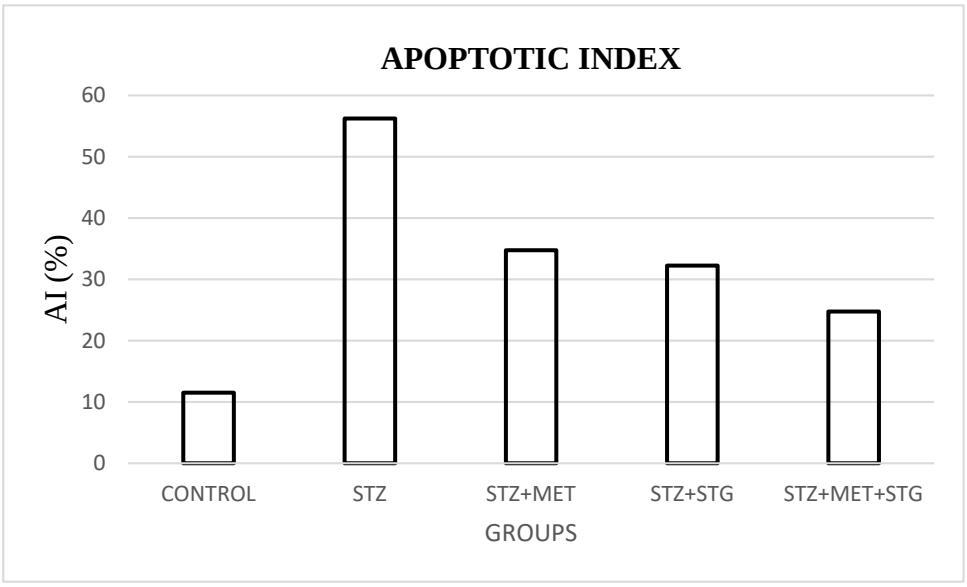


Figure 3. Apoptotic index of groups in liver tissues.

DISCUSSION

The STZ diabetogenic effect is caused by the excess production of ROS, which result in the toxicity of pancreatic cells, affecting organs such as the kidney, liver, and hematopoietic system[27-29]. The present study illustrated that the effect of metformin and sitagliptin against oxidative stress in diabetic rats' liver tissue.

Lipid peroxidation begins with the attack of free radicals on membrane lipids. Increased levels of lipid peroxidation may have played a role in diabetes-related tissue damage[30,31]. According the results of this study, the lipid peroxidation index of MDA formation was significantly increased in the liver of animals with STZ-diabetes. These results we obtained are consistent with previous studies[32,33].

Oxidative stress means the lack of balance between the cells' antioxidant capacity and the production of ROS. Increased oxidative stress, which contributes significantly to the pathogenesis of diabetic complications, results from either increased ROS production or decreased antioxidant capacity[34,35].

Regarding the effect of current diabetic pharmacotherapy in the present study, metformin and sitagliptin might affect the oxidant and antioxidant status. In rat liver tissue, MDA, TAS, TOS, and OSI of metformin plus sitagliptin administered group returned normal levels (table 1.). Metformin inhibits oxidative phosphorylation, reducing cellular ATP levels, production of ROS, and induction of DNA repair[36]. Sitagliptin and other DDP4 inhibitors prevent hyperglycemia-induced OS with a significant antioxidant effect by reversing ROS and endoplasmic reticulum stress[37]. Literature data have shown that sitagliptin can be applied in the form of monotherapy, as it does not interact with other antidiabetic drugs, it can also be used in combination therapies[38]. Studies conducted to determine the relationship between sitagliptin and oxidative stress stated that sitagliptin does not affect serum MDA levels from oxidative parameters but increases oxidative stress in serum by decreasing total antioxidant level (TAS)[39].

Further studies should be done to determine the effect of oxidative stress in diabetes using another antidiabetic agent[40]. Several studies have shown the effect of oxidative stress on the development of diabetes mellitus. Free radicals are produced along with hyperglycemia and liver dysfunction, leading to a significant reduction in hepatic antioxidants[41].

The liver is a central metabolic organ and it has been reported that it is exposed to reactive oxygen species formed due to oxidative damage resulting from

diabetes[42]. For this reason, it has been suggested that apoptosis is formed in hepatocytes and endothelial cells[43]. In STZ-induced diabetes studies, necrosis of hepatocytes, inflammatory cell infiltration, lipidosis[44], dilatation of sinusoids and impairment in the portal spaces reported[45]. In the present study, similar findings were observed in diabetic rats. It was found that these shaped findings decreased significantly with MET + STG treatment. Changes in the livers of the groups treated with metformin and sitagliptin alone were found to be better than the histopathological picture associated with diabetes complications. It was observed that a much more effective treatment was provided in the groups treated with metformin and sitagliptin combined therapy. Especially liver histology has become close to normal.

The effects of diabetes on the liver are ultrastructural changes such as autophagic vacuoles and hypertrophy in hepatocytes. The hepatocyte nucleus usually has an enlarged appearance, sometimes with intranuclear inclusions and irregular contours. In the diabetic group, cytoplasmic changes are seen as reduced glycogen granules and underdeveloped granular endoplasmic reticulum[46]. In our study, widespread degeneration was observed in hepatocytes of diabetic rats under light microscopy. The perisinusoidal fibrous and collagenous material increase was not observed in any group. A previous study reported fibrosis in the diabetic liver [46]. However, some authors claimed that there was no direct relationship between the fibrosis in the diabetic liver and diabetes, but it is caused by liver vascular anomalies and genetic predisposition in the studied rat strain[47]. The findings on the liver in our study showed that fibrosis did not show a severe spread, but hepatocyte degeneration occurred with nuclear changes. In particular, cytoplasmic vacuolization was similar to the findings in other studies. When the literature information was examined, it was determined that the production of free oxygen radicals was accelerated in STZ-induced diabetic rats and type 1 and type 2 diabetes patients. Along with the increase in the lipid peroxidation level caused by increased oxidative stress, impairments in glutathione homeostasis are also detected. These redox status changes were observed in the erythrocytes of rats treated with STZ and of both types of diabetes patients. Melatonin administration is effective in protecting glutathione homeostasis disorders that occur with diabetes. After melatonin administration, an increase in diabetic animals' GSH level has been shown in the kidney, liver, brain, heart tissues and plasma[48-50]. It was determined that our findings were compatible with the type 1 diabetes model created by STZ and that biochemical parameters increased with oxidative stress. It was also confirmed by the TUNEL technique that tissue damage triggers apoptosis with oxidative stress. It was observed that apoptosis decreased at the highest level with MET + STG treatment.

The limitation of this study, with a low dosage of sitagliptin and metformin used herein, more caution should be exercised in the dosage used in humans. Such limitations should be addressed in future clinical trials.

In conclusion, this study showed that combination therapy with metformin and sitagliptin could effectively protect against oxidative damage in liver tissue of rats with STZ-induced diabetes. The enzymatic and non-enzymatic antioxidant defense system could be improved, and lipid peroxidation could be prevented using this combined therapy. These observations suggest that a preferable drug(s) for diabetic patients are sitagliptin or metformin. Further studies should be done for clarification of the mechanism and potential of antidiabetic drugs.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SÇ, ŞÖ; Veri toplama: SÇ, ŞÖ; Veri analizi ve yorumlama: SÇ, ŞÖ; Yazı taslağı: SÇ, ŞÖ; İçeriğin eleştirel incelenmesi: SÇ, ŞÖ; Son onay ve sorumluluk: SÇ, ŞÖ; Teknik ve malzeme desteği: SÇ, ŞÖ; Süpervizyon: SÇ; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Çanakkale Onsekiz Mart Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan 20.04.2020 tarih ve 2020/04-04 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Author Contributions: Concept/Design : SÇ, ŞÖ; Data acquisition: SÇ, ŞÖ; Data analysis and interpretation: SÇ, ŞÖ; Drafting manuscript: SÇ, ŞÖ; Critical revision of manuscript: SÇ, ŞÖ; Final approval and accountability: SÇ, ŞÖ; Technical or material support: SÇ, ŞÖ; Supervision: SÇ; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained for this study from Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee with the decision dated 20.04.2020 and numbered 2020 / 04-04.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. Mohamed J, Nazratun Nafizah AH, Zariyantey AH, Budin SB. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. Sultan Qaboos Univ Med J. 2016;16:132-41.
2. Shawky LM, Morsi AA, El Bana E, Hanafy SM. The biological impacts of sitagliptin on the pancreas of a rat model of type 2 diabetes mellitus: drug interactions

with metformin. Biology (Basel). 2019;9:6.
3. Alam MM, Ahmad I, Naseem I. Inhibitory effect of quercetin in the formation of advance glycation end products of human serum albumin: An in vitro and molecular interaction study. Int J Biol Macromol. 2015;79:336-43.
4. Eitah HE, Maklad YA, Abdelkader NF, Gamal El Din AA, Badawi MA, Kenawy SA. Modulating impacts of quercetin/sitagliptin combination on streptozotocin-induced diabetes mellitus in rats. Toxicol Appl Pharmacol. 2019;365:30-40.
5. Baig NA, Herrine SK, Rubin R. Liver disease and diabetes mellitus. Clin Lab Med. 2001;21:193-207.
6. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress: A concise review. Saudi Pharm J. 2016;24:547-53.
7. Bansal AK, Bilaspuri GS. Impacts of oxidative stress and antioxidants on semen functions. Vet Med Int. 2010;2010:686137.
8. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5:9-19.
9. Hostalek U, Gwilt M, Hildemann S. therapeutic use of metformin in prediabetes and diabetes prevention. Drugs. 2015;75:1071-94.
10. Algire C, Moiseeva O, Deschênes-Simard X, Amrein L, Petruccelli L, Birman E et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. Cancer Prev Res (Phila). 2012;5:536-43.
11. Carvalho C, Correia S, Santos M, Seiça R, Oliveira C, Moreira P. Metformin promotes isolated rat liver mitochondria impairment. Mol Cel Biochem. 2008;308:75-83.
12. Kelly B, Tannahill GM, Murphy MP, O'Neill LA. Metformin inhibits the production of reactive oxygen species from NADH:ubiquinone oxidoreductase to limit induction of interleukin-1β (IL-1β) and Boosts Interleukin-10 (IL-10) in lipopolysaccharide (LPS)-activated macrophages. J Biol Chem. 2015;290:20348-59.
13. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000;348:607-14.
14. Chen YT, Tsai TH, Yang CC, Sun CK, Chang LT, Chen HH et al. Exendin-4 and sitagliptin protect kidney from ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction. J Transl Med. 2013;11:270.
15. Kumar A, Pathak R, Palfrey HA, Stone KP, Gettys TW, Murthy SN. High levels of dietary methionine improves sitagliptin-induced hepatotoxicity by attenuating oxidative stress in hypercholesterolemic rats. Nutr Metab. 2020;17:2.
16. Trocha M, Krzystek-Korpacka M, Merwid-Ląd A, Nowak B, Pieśniewska B, Dzięgiel P et al. Sitagliptin-dependent differences in the intensity of oxidative stress in rat livers subjected to ischemia and reperfusion. Oxid Med Cell Longev. 2019;2019:2738605.
17. Vaghasiya J, Sheth N, Bhalodia Y, Manek R. Sitagliptin protects renal ischemia reperfusion induced renal damage in diabetes. Regul Peptides. 2011;166:48-54.
18. National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A. The National Academies Collection: Reports funded by National Institutes of Health. Guide for the Care and Use of Laboratory Animals. Washington (DC), National Academy of Sciences, 2011.
19. Wu KK, Huan Y. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2008;5:47.
20. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38:1103-11.
21. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37:277-85.
22. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly. 2003;133:563-66.
23. Kosecik M, Erel O, Sevinc E, Selek Ş. Increased oxidative stress in children exposed to passive smoking. Int J Cardiol. 2005;100:61-4.
24. Yumru M, Savas HA, Kalenderoglu A, Bulut M, Celik H, Erel O. Oxidative imbalance in bipolar disorder subtypes: a comparative study. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33:1070-4.
25. Li CJ, Yang ZH, Shi XL, Liu DL. Effects of aspirin and enoxaparin in a rat model of liver fibrosis. World J Gastroenterol. 2017;23:6412-9.
26. Soini Y, Pääkkö P, Lehto VP. Histopathological evaluation of apoptosis in cancer. Am J Pathol. 1998;153:1041-53.
27. Al-kuraishy H. Oxidative stress injury and glucolipotoxicity in type 2 diabetes mellitus: the potential role of metformin and sitagliptin. Biomed Res Int. 2020;4:166-72.
28. Derosa G, D'Angelo A, Maffioli P. Sitagliptin in type 2 diabetes mellitus: Efficacy after five years of therapy. Pharmacol Res. 2015;100:127-34.
29. Yabe D, Kuwata H, Kaneko M, Ito C, Nishikino R, Murorani K et al. Use of the Japanese health insurance claims database to assess the risk of acute pancreatitis in patients with diabetes: comparison of DPP-4 inhibitors with other oral antidiabetic drugs. Diabetes Obes Metab. 2015;17:430-34.
30. Arora R, Vig A, Arora S. Lipid peroxidation: a possible marker for diabetes. J Diabetes Metab. 2013;SII:007.
31. Elsawy M, Emara E. The impact of ghrelin on oxidative stress and inflammatory markers on the liver

of diabetic rats. Tanta Medical Journal. 2016;44:163-9.
32. Emara E, Elsawy M, Elmashad W, Eldamarawi M. Effect of vitamins (C and E) on endothelial inflammation biomarkers and oxidative stress in diabetic rats. J Appl Sci. 2014;14:3563-70.
33. Gezginci-Oktayoglu S, Basaraner H, Yanardag R. The effects of combined treatment of antioxidants on the liver injury in STZ diabetic rats. Dig Dis Sci. 2008;54:538-46.
34. Koyuturk M, Sacan O, Karabulut S, Turk N, Bolkent S, Yanardag R et al. The role of ghrelin on apoptosis, cell proliferation and oxidant-antioxidant system in the liver of neonatal diabetic rats. Cell Biol Int.. 2015;39:834-41.
35. Parveen K, Khan MR, Mujeeb M, Siddiqui WA. Protective effects of Pycnogenol on hyperglycemia-induced oxidative damage in the liver of type 2 diabetic rats. Chem Biol Interact. 2010;186:219-27.
36. Dogan I, Çandar T, Yuksel E, Kalay S, Oğuz AK, Demirtas S. Potential effects of metformin in DNA BER system based on oxidative status in type 2 diabetes. Biochimie. 2018;154:62-8.
37. Pujadas G, De Nigris V, Prattichizzo F, La Sala L, Testa R, Ceriello A. The dipeptidyl peptidase-4 (DPP-4) inhibitor teneligliptin functions as antioxidant on human endothelial cells exposed to chronic hyperglycemia and metabolic high-glucose memory. Endocrine. 2017;56:509-20.
38. Mistry GC, Bergman AJ, Zheng W, Hreniuk D, Zinny MA, Gottesdiener KM et al. Sitagliptin, an dipeptidyl peptidase-4 inhibitor, does not alter the pharmacokinetics of the sulphonylurea, glyburide, in healthy subjects. Br J Clin Pharmacol. 2008;66:36-42.
39. Ferreira L, Teixeira-de-Lemos E, Pinto F, Parada B, Mega C, Vala H et al. Effects of sitagliptin treatment on dysmetabolism, inflammation, and oxidative stress in an animal model of type 2 diabetes (ZDF rat). Mediators Inflamm. 2010;2010:592760.
40. Monami M, Iacomelli I, Marchionni N, Mannucci E. Dipeptydil peptidase-4 inhibitors in type 2 diabetes: a meta-analysis of randomized clinical trials. Nutr Metab Cardiovasc Dis. 2010;20:224-35.
41. Hamden K, Carreau S, Boujbiha MA, Lajmi S, Aloulou D, Kchaou D et al. Hyperglycaemia, stress oxidant, liver dysfunction and histological changes in diabetic male rat pancreas and liver: protective effect of 17 beta-estradiol. Steroids. 2008;73:495-501.
42. Seven A, Guzel S, Seymen O, Civelek S, Bolayirli M, Uncu M et al. Effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: investigation of liver and plasma. Yonsei Med J. 2004;45:703-10.
43. Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury. J Gastroenterol Hepatol. 2000;15:718-24.
44. Zhang C, Lu X, Tan Y, Li B, Miao X, Jin L et al. Diabetes-induced hepatic pathogenic damage, inflammation, oxidative stress, and insulin resistance was exacerbated in zinc deficient mouse model. PloS One. 2012;7:e49257.
45. Noor A, Gunasekaran S, Amirtham S, Vijayalakshmi MA. Antidiabetic activity of Aloe vera and histology of organs in streptozotocin-induced diabetic rats. Curr Sci. 2008;94:1070-76.
46. Kume E, Fujimura H, Matsuki N, Ito M, Aruga C, Toriumi W et al. Hepatic changes in the acute phase of streptozotocin (SZ)-induced diabetes in mice. Exp Toxicol Pathol. 2004;55:467-80.
47. Brůha R, Dvořák K, Petrtýl J. Liver disorders in diabetic patients. Vnitrni lekarstvi. 2013;59:546-50.
48. Aksoy N, Vural H, Sabuncu T, Aksoy S. Effects of melatonin on oxidative antioxidative status of tissues in streptozotocin-induced diabetic rats. Cell Biochem Funct. 2003;21:121-5.
49. Baydas G, Reiter R, Yasar A, Tuzcu M, Akdemir I, Nedzvetsky V. Melatonin reduces glial reactivity in the hippocampus, cortex, and cerebellum of streptozotocin-induced diabetic rats. Free Radic Biol Med. 2003;35:797-804.
50. Winiarska K, Fraczyk T, Malinska D, Drozak J, Bryla J. Melatonin attenuates diabetes-induced oxidative stress in rabbits. J Pineal Res. 2006;40:168-76.