İSTANBUL UNIVERSITY PRESS

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE
Journal of Advanced Research in Health Sciences /
Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2022
DOI: 10.26650/JARHS2022-1105948

PROTECTIVE EFFECT OF MITOTEMPO IN STREPTOZOTOCIN-INDUCED DIABETIC RAT MODEL: EFFECTS ON CORPUS CAVERNOSUM AND AORTA

STREPTOZOTOSİN İLE İNDÜKLENEN DİYABETLİ SIÇAN AORT VE KORPUS KAVERNOZUM DOKULARINDA MİTOTEMPO'NUN ENDOTEL ÜZERİNE MUHTEMEL KORUYUCU ETKİSİ

Eser YILDIZ[1], Pınar AYVAT[2], Ayşe Saide ŞAHİN[1], Mehmet Mesut PİŞKİN[3], Burak Cem SONER[4]

[1]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Medical Pharmacology, Konya, Turkiye
[2]Izmir Democracy University, Faculty of Medicine, Department of Anesthesiology, Izmir, Turkiye
[3]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Urology, Konya, Turkiye
[4]Izmir Democracy University, Faculty of Medicine, Department of Pharmacology, Izmir, Turkiye

ORCID ID: E.Y. 0000-0001-9321-1066; P.A. 0000-0002-9941-3109; A.S.Ş. 0000-0001-6432-5822; M.M.P. 0000-0002-0528-6699; B.C.S. 0000-0002-3712-3210

Citation/Atıf: Yildiz E, Ayvat P, Sahin AS, Piskin MM, Soner BC. Protective effect of mitotempo in streptozotocin-induced diabetic rat model: Effects on corpus cavernosum and aorta. Journal of Advanced Research in Health Sciences 2022;5(2):82-87. https://doi.org/10.26650/JARHS2022-1105948

ABSTRACT

Objective: In this study we aimed to evaluate the effect of mitotempo, a mitochondria-specific antioxidant, on endothelial and erectile dysfunction in a Streptozotocin-induced rat diabetes model.
Material and Methods: Wistar Albino rats weighing 280-320 g were used in the study. Diabetes was induced by intraperitoneal 50 mg/kg single dose streptozotocin injection. Blood glucose levels above 300 mg/dl at the end of 1 week were considered diabetes. Blood glucose was monitored for 4 weeks. The treatment group received mitotempo orally at 0.7 mg/kg/day for 4 weeks. At the end of the 4th week, the aorta and corpus cavernosum contraction and relaxation responses were evaluated in an isolated organ bath after decapitation.
Results: According to our study results, mitotempo 0.7 mg/kg/day for 4 weeks in a diabetes model preserved endothelial relaxation responses in both the thoracic aorta and corpus cavernosum. Phenylephrine contractions calculated according to KCl contraction did not differ between the groups.
Conclusion: Endothelial cells can be identified as one of the first organs to be exposed to circulating substances. The effects of mROS on endothelial dysfunction caused by hyperglycemia is known. In our study, we found that a 0.7 mg/kg/day mitotempo treatment for 4 weeks showed protective effects on STZ-induced diabetic endothelial dysfunction.
Keywords: Mitotempo, Aorta, Corpus Cavernosum, Diabetes

ÖZ

Amaç: Deneysel olarak sıçanlarda Streptozotosin ile oluşturulmuş diyabet modelinde bir mitokondri spesifik antioksidan olan Mitotempo'nun endotelial ve erektil disfonksiyona etkisinin incelenmesi amaçlanmıştır.
Gereç ve Yöntem: Deney için 280-320 g ağırlığında Wistar-Albino soyu sıçanlar kullanılmıştır. Diyabet grubuna 50 mg/kg intraperitoneal tek doz streptozotosin uygulanmıştır. 1 hafta sonunda 300 mg/dl kan glikozu seviyesinin üstünde olanlar diyabet kabul edilmiştir. 4 hafta boyunca kan glikozu takip edilmiştir. Bu 4 hafta boyunca tedavi grubuna 0.7 mg/kg/gün oral mitotempo uygulanmıştır. 4. Hafta sonunda dekapite edilen hayvanların aort ve korpus kavernozum dokuları alınarak kasılma ve gevşeme yanıtları izole organ banyosunda değerlendirilmiştir.
Bulgular: Çalışma sonuçlarımıza göre diyabet modelinde mitotemponun 0.7 mg/kg/gün dozunda 4 hafta süre ile uygulanması hem torasik aorta hem de korpus kavernosum endotel yanıtlarını korumuştur. KCl kasılmasına göre rölatif olarak değerlendirilen fenilefrin kasılma yanıtları gruplar arasında farklılık göstermemiştir.
Sonuç: Endotel hücreleri, dolaşımdaki maddelere ilk maruz kalan organlardan biri olarak tanımlanabilir. Hipergliseminin neden olduğu endotel disfonksiyonda mROS'un etkisi bilinmektedir. Diyabete bağlı oluşan endotel disfonksiyonun engellenebilmesi hastalığa bağlı mortalite ve morbiditenin önüne geçebilir. Çalışmamızda mitotemponun diyabetik endotelial disfonksiyon üzerine olası etkileri değerlendirilmiş ve sınırlı koruyucu etkileri gösterilmiştir.
Anahtar Kelimeler: Mitotempo, Aort, Korpus Kavernosum, Diyabet

Corresponding Author/Sorumlu Yazar: Burak Cem SONER **E-mail:** burakcemsoner@gmail.com
Submitted/Başvuru: 21.04.2022 • **Revision Requested/Revizyon Talebi:** 25.04.2022 • **Last Revision Received/Son Revizyon:** 18.05.2022 • **Accepted/Kabul:** 20.05.2022 • **Published Online/Online Yayın:** 22.06.2022

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder; microvascular and macrovascular complications of DM lead to disorders in various organs. Endothelial dysfunction means structural and/or functional disability in endothelial responses, which is an important factor in cardiovascular disorders (CVD). Endothelial dysfunction has been accepted as the initial step of atherosclerosis. Erectile dysfunction (ED) is an early marker of CVD because the initial and main pathology that underlies erectile dysfunction is also accepted as endothelial dysfunction (1-3). Oxidative stress triggered by hyperglycemia is related to ED (4). Some studies have shown that mitochondrial and nonmitochondrial antioxidant agents can prevent endothelial disfunction (5). Animal models of diabetes have been accepted as proper models for erectile dysfunction and have been suggested for preclinical studies for further insight into prevention and treatment (6).

Mitochondria-targeted antioxidants are newly developed synthetic vehicles used for oxidative stress related pathologies (7). Previously studied antioxidants have also been adapted to mitochondrial antioxidant delivery systems for this purpose (8).

To create these agents, some additional complex structures are used. For example, 2,2,6,6-tetramethylpiperidine-1-oxy (Tempo), a piperidine nitroxide derivative, is a protective radical in various animal and cell models such as oxidative stress, aging, and degenerative disorders (9). These effects are often attributed to the antioxidant reactions of cyclic nitroxides. One of the proper mitochondria-specific antioxidants is mitotempo. Antioxidant effects of mitotempo have been shown in various studies such as on age-related arterial endothelial dysfunction and preeclampsia (10, 11).

In this study we aimed to show the endothelial protective effects of mitotempo on STZ-induced animal diabetes model in thoracal aorta (TA) and corpus cavernosum (CC) tissues, both of which have been proven to be the initiation of vascular complications and erectile dysfunction, respectively.

MATERIALS AND METHODS

Our study is approved by the Konya Necmettin Erbakan University Animal Care and Use Committee (application number 2018.034). Male Wistar Albino rats weighting 280-320 g were procured and separated into 3 groups: Control, Diabetes and Diabetes+Mitotempo.

The Control Group did not undergo any treatment procedure. Diabetes was induced by intraperitoneal injection of a single dose of 50 mg/kg of Streptozotocin (STZ) (12). The Diabetes and Diabetes+Mitotempo groups received 50 mg/kg of STZ intraperitoneally. One week after this procedure, blood glucose levels were measured, and values over 300 mg/dl were accepted as diabetes. While the Control and Diabetes groups received distilled water, the Diabetes + Mitotempo group received 0.7 mg/kg/day mitotempo via oral gavage for 4 weeks. Weekly blood glucose values were measured for all groups. After 4 weeks, the animals were decapitated. TA and CC tissues were isolated for the organ bath experiment.

Isolated Organ Bath Experiment Protocol:
After sacrification, TA and CC tissues were removed. Tissues were enclosed in +4°C Krebs-Henseleit Solution (KHS). The excised TA was cleared from connective tissues without causing endothelial damage, and ring-shaped preparations 2-3 mm wide were prepared. Silk threads were applied to both ends of the preparations. Using these silk threads, one end was suspended on fixed metal; the other end was suspended on the isometric transducer. The preparations were hung in the organ bath that contained 37°C KHS and was gasified with a 95% O_2+5% CO_2 mixture. The excised CC tissue also underwent the same procedures. Both tissues (TA and CC) were rested at 37°C and under 1 g tension by changing the Krebs solution every 15 minutes per hour. Isometric tensions were recorded with an amplifier system (MP30 Biopac systems Inc., Santa Barbara, CA, USA) on a computer using the Biopac program.

After a 30 minute-resting period, tissues were contracted with 80 mM KCl. After the contraction responses reached a plateau, the bath was refilled with 10 ml KHS and the tissues were allowed to rest for 30 minutes (KHS was changed every 15 min). After this 30 minute-resting period, 10^{-6} M phenylephrine (PE) submaximal contraction responses were examined. When the contraction responses reached the plateau phase, Acetylcholine (Ach) was added cumulatively to the organ bath (log molar, 10^{-9} to 10^{-5} M) and endothelial relaxation responses were evaluated.

Chemicals Used: Acetylcholine HCl, (Product Number A6625); Phenylephrine HCl (Product Number P6126); Mitotempo (Product Number SML0737) and Streptozotocin (Product Number S0130) obtained from Sigma Aldrich, (St. Louis, MO, U.S.)

Krebs Henseleit solution (mM):
NaCl 119, KCl 4.6 $CaCl_2$ 1.5 MgCl 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 5.5 pH: 7.4 and 80 mM KCl solution (mM): NaCl 43.6, KCl 80, CaCl2 1.5, MgCl 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 5.5 pH: 7.4 had the following composition. All chemicals for Krebs solution were obtained from Merck KGaA (Darmstadt, Germany).

Data Analysis:
Contraction responses after the PE application were calculated as a percentage of KCl responses. As for relaxant agent responses, the submaximal response with PE application was taken as 100% and Ach responses were calculated as a percentage of this value. A comparison of data suitable for normal distribution was made using the independent t-test (Unpaired Student's t-test), while the comparison of data not compatible with normal distribution was made using the Mann-Whitney U test. The data with a p-value of less than 0.05 were considered statistically significant. At the end of the study, all group responses were compared. P-values of 0.05 were considered significant.

RESULTS

In our results, PE contraction responses showed no statistical significance between Control, Diabetes, and Diabetes+Mitotempo groups both for TA and CC tissue in contraction responses with PE. Comparison of Ach-induced relaxation responses showed a statistically significant difference between diabetes and Diabetes+Mitotempo groups. Ach relaxation responses of the Diabetes+Mitotempo group were higher than the Diabetes Group, which showed that mitotempo had a protective effect against endothelial dysfunction and ED that are caused by diabetes.

Blood Glucose Levels: Blood glucose levels of experimental animals were measured at 1-week intervals after STZ administration and values above 300 mg/dl were accepted as diabetes (13). Average weekly blood glucose values are given in Figure 1. Although blood glucose levels of treated diabetic rats seem to be slightly lower than the Diabetes Group, no statistical differences was observed.

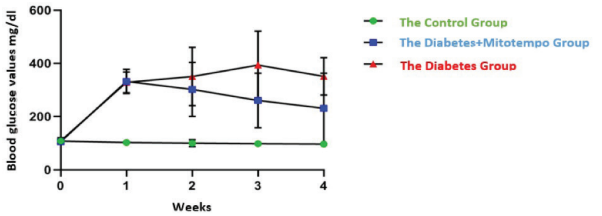

Figure 1: Blood glucose values measured weekly

Contraction Responses of Aorta Tissue with Phenylephrine: In our test protocol, 10^{-6}M PE contraction responses were evaluated as a percentage of 80 mM KCl contraction. The contraction response obtained from the Control Group (n=6) was determined to be 149.93±29.86%. In the Diabetes Group (n=6), PE responses were obtained as 118.13±19.13%, whereas in the Diabetes+Mitotempo Group (n=6), these results were observed as 126.75±5.46%. In the comparison of 10^{-6}M PE contraction responses between groups, no significant difference was observed between the groups ($p > 0.05$) (Figure 2).

Relaxation Responses of Aorta Tissue with Acetylcholine after Phenylephrine Contraction: During the test protocol, 10^{-9}M-10^{-5}M cumulative effect of Ach was evaluated after 10^{-6} M PE responses reached the plateau phase. Ach maximum relaxation responses were 69.9±29.9% in the Control Group, 34.49±4.04% in the Diabetes Group and 54.76±36.88% in the Diabetes+Mitotempo Group. Maximum relaxation responses obtained with Ach were evaluated as a percentage of 10^{-6} M PE response; a statistically significant difference was observed between the Diabetes Group when compared with the Control and Diabetes+Mitotempo Groups ($p < 0.05$) (Figure 3).

Contraction Responses of Corpus Cavernosum Tissue with Phenylephrine: In our experiment protocol, 10^{-6}M PE contraction responses were evaluated as a percent over basal contraction responses with 80 mM KCl contraction.

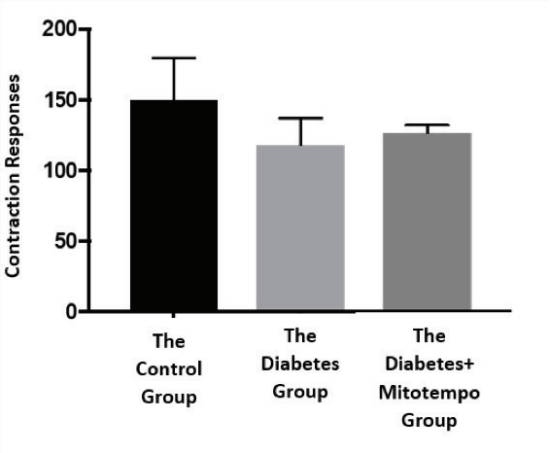

Figure 2: Aortic PE contraction responses of the Control Group, the Diabetes Group, and Diabetes+Mitotempo Group. Contraction responses after the PE application were calculated as a percentage of KCl responses.

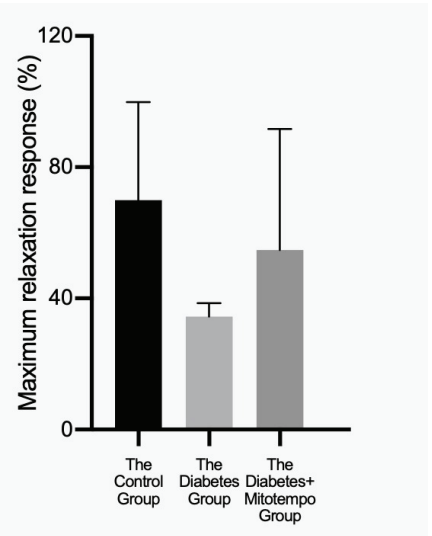

Figure 3: Maximum relaxation response of Aorta to acetylcholine. Ach responses were calculated as a percentage of the submaximal dose PE application (10-6 M). * $p < 0.05$

Contraction responses of the Control Group (n=6) were determined to be 101.83±5.52%. In the Diabetes Group (n=6), PE responses were obtained as 93.15±4.34%, while in the Diabetes+Mitotempo Group (n=6), these results were observed as 92.34±4.78%. No significant difference was observed between the groups ($p > 0.05$) (Figure 4).

Relaxation Responses of Corpus Cavernosum with Acetylcholine after Phenylephrine Contraction: Cumulative 10^{-9} M to 10^{-5} M Ach effect in the contraction response that reached the plateau phase after 10^{-6} M PE application was evaluated. Ach maximum relaxation responses were 78.74±12.18% in the Control Group, 32.47±15.85% in the Diabetes Group and 63.16±24.78% in the Diabetes+Mitotempo Group. When the maximum relaxation responses obtained with Ach were evaluated as a percentage

of 10^{-6} M PE response, a significant difference was observed in the Diabetes Group versus Control and Diabetes+Mitotempo Group ($p < 0.05$) (Figure 5).

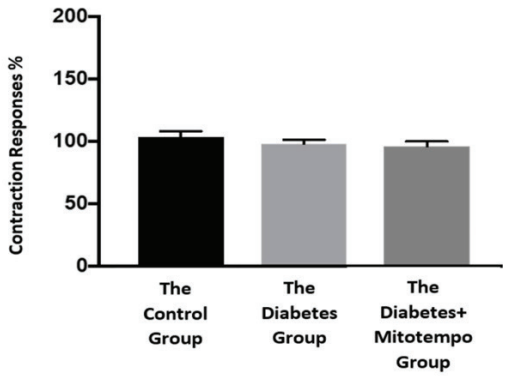

Figure 4: PE contraction responses of corpus cavernosum tissue. Contraction responses after the PE application were calculated as a percentage of KCl responses.

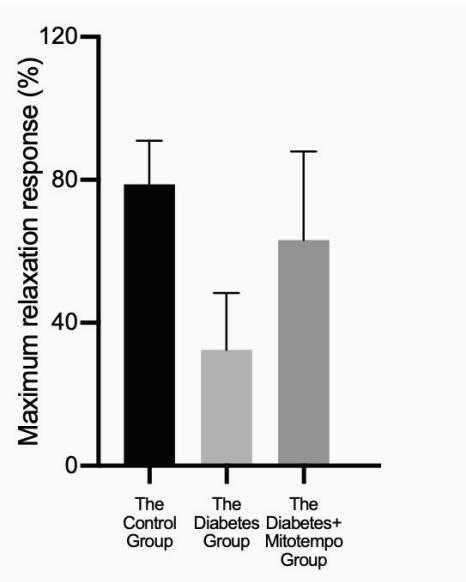

Figure 5: Maximum relaxation response of corpus cavernosum to acetylcholine. Ach responses were calculated as a percentage of the submaximal dose PE application (10-6 M). * $p<0.05$

DISCUSSION

Endothelial dysfunction and ED treatment have been investigated for years. Pharmaceutical products aim to reduce/regress ED-related symptoms. Antioxidant treatments have been tried in many disease models for many years. Antioxidants seem to have protective effects on endothelial damage in animal model of diabetes both for CC and vascular endothelium (5). On the other hand, in clinical research, the protective efficacy of any antioxidants is yet to be proven. One of the reasons for this ineffectiveness of antioxidants in treatment is the fact that the antioxidants applied cannot affect the inner cell, such as mitochondria organelle, and therefore cannot have a protective effect on pathological oxidative damage (14-16). Researchers have been showing interest in mitochondrial pharmacology and research on the subject is increasing every day.

Many diseases, such as atherosclerosis, DM, coronary artery diseases, hypertension, and hypercholesterolemia cause ED. The development of ED in these diseases has been associated with mitochondrial dysfunction. Today, mROS is held responsible for ED caused by diabetes (14).

The antihypertensive effect of mitotempo has been studied in the hypertension mouse model. In this study, it is shown that Angiotensin II (ATII) increases endothelial mitochondrial superoxide production, and mitotempo therapy reverses this effect. The results of the study showed that mitotempo therapy was effective in the early and late periods of the treatment of hypertension induced by AT II and deoxycorticosterone acetate (DOCA)-salt (17). There are many studies targeting the formation of mitochondrial superoxide in the treatment of hypertension (18). These studies suggest that if hypertension occurs after the formation of mitochondrial superoxide, mitotempo therapy can reverse or improve this endothelial cell stress. In studies conducted, it was stated that this antihypertensive effect was dose-dependent (17).

Although atherosclerosis is frequently seen after hyperlipidemia, diabetes is also known as a risk factor for atherosclerosis. In a study with APO E knockout mice known to be suitable for the atherosclerosis model, mitotempo has been shown to prevent endothelial cell activation and monocyte migration into the aorta. The event described here as "endothelial cell activation" is indicated as an initiator of atherosclerosis/ED. Types of mitochondrial reactive oxygen are thought to activate this cascade. Mitotempo, on the other hand, appears to prevent atherosclerosis by acting in this "initial" stage (19).

McCarthy et al. studied the effects of mitotempo on preeclampsia. Preeclampsia causes vascular dysfunction through mitochondrial functions, as in DM. In this study, pretreatment with mitotempo, which is a mitochondria-targeted antioxidant, prevented cell death caused by 200 µM H_2O_2 and showed successful results in the preeclampsia model (11).

In our study, the effects of mitotempo treatment on endothelial dysfunctions of the aorta and corpus cavernosum in the diabetes model created by STZ in Wistar rats were evaluated. Our results have shown the limited protective effect of 0.7 mg/kg mitotempo treatment over a period of 4 weeks in Wistar rats in hyperglycemia-related endothelial dysfunction. This protective effect of mitotempo cannot be correlated with blood glucose levels because, like previous studies, our study also showed no significant effect of mitotempo on blood glucose levels in diabetic animal models (20, 21). It is known that many mitochondrial and non-mitochondrial oxidant systems have effects on endothelium. Our study, for the first time, evaluated the effects of the mitochondrial antioxidant system on corpus cavernosum with mitotempo treatment in rat diabetes model.

In our study we have evaluated the effects of only a 0.7 mg/kg/day mitotempo treatment. This can be considered a limitation of our study, since the effects of a higher dose have not been demonstrated. In our dose selection we extrapolated the dosage of previous studies. Previously, Dikalova et al. has shown the endothelial protective effect of mitotempo in angiotensin II and DOCA-salt induced hypertensive mice model. In this study, mitotempo was administered at 0.7 mg/kg/day for seven days (17). Ni et al. also studied the protective effects of mitotempo on STZ-induced cardiomyopathy in a diabetic mice model with a dose of 0.7 mg/kg/day for 30 days. Their results showed that mitochondrial ROS inhibition improved myocardial function in a mice diabetes model (20). Higher doses of mitotempo have also been studied. In a coronary endothelial dysfunction model, 1 mg/kg/day for 4 weeks has been studied in a diabetes model. In this study, the protective effects of mitotempo have been shown in coronary endothelial dysfunction.

In conclusion, endothelial cells can be defined as one of the organs first exposed to circulating substances. The effect of mROS is known in endothelial dysfunction induced by hyperglycemia. As stated, endothelial dysfunction is the initiation of both vascular diseases and ED. In our study, we obtained beneficial results on diabetes-induced ED by using the mitochondrial antioxidant mitotempo. This effect of mitotempo may be due to the reduction of mROS production. Further studies should be conducted to fully clarify the mechanism and the maximum effective dose selection for the protective effect of mitotempo on ED and endothelium.

Ethics Committee Approval: This study is approved by Konya Necmettin Erbakan University Animal Care and Use Committee. (Date: 19.10.2018, No: 2018-034.)

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study-E.Y., B.C.S., M.M.P., A.S.Ş.; Data Acquisition- E.Y., B.C.S., A.S.Ş.; Data Analysis/Interpretation- E.Y., B.C.S., A.S.Ş., M.M.P., P.A.; Drafting Manuscript- E.Y., B.C.S., P.A.; Critical Revision of Manuscript- B.C.S., P.A., M.M.P; Approval and Accountability- E.Y., B.C.S., P.A., M.M.P., A.S.Ş.; Material and Technical Support- E.Y., B.C.S., M.M.P., A.S.Ş.; Supervision- E.Y., B.C.S., M.M.P., A.S.Ş.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Necmettin Erbakan Üniversitesi KONÜDAM Deneysel Tıp Uygulama ve Araştırma Merkezi Müdürlüğü Etik Kurulu'ndan alınmıştır (Tarih: 19.07.2019, No: 2019.204. IRB1.027).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- E.Y., B.C.S., M.M.P., A.S.Ş.; Veri Toplama- E.Y., B.C.S., A.S.Ş.; Veri Analizi/Yorumlama- E.Y., B.C.S., A.S.Ş., M.M.P., P.A.; Yazı Taslağı- E.Y., B.C.S., P.A.; İçeriğin Eleştirel İncelemesi- B.C.S., P.A., M.M.P; Son Onay ve Sorumluluk- E.Y., B.C.S., P.A., M.M.P., A.S.Ş.; Malzeme ve Teknik Destek- E.Y., B.C.S., M.M.P., A.S.Ş.; Süpervizyon- E.Y., B.C.S., M.M.P., A.S.Ş.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Goldstein I, Young JM, Fischer J, Bangerter K, Segerson T, Taylor T, Vardenafil Diabetes Study Group et al. Vardenafil, a new phosphodiesterase type 5 inhibitor, in the treatment of erectile dysfunction in men with diabetes: A multicenter double-blind placebo-controlled fixed-dose study. Diabetes Care 2003;26(3):777-83.
2. Palumbo PJ. Metabolic risk factors, endothelial dysfunction, and erectile dysfunction in men with diabetes. Am J Med Sci 2007;334(6):466-80.
3. Vlachopoulos C, Ioakeimidis N, Terentes-Printzios D, Stefanadis C. The Triad: Erectile Dysfunction - Endothelial Dysfunction - Cardiovascular Disease. Curr Pharm Des 2008;14(35):3700-14.
4. Azadzoi KM, Schulman RN, Aviram M, Siroky MB. Oxidative stress in arteriogenic erectile dysfunction: Prophylactic role of antioxidants. J Urol 2005;174(1):386-93.
5. Soner BC, Murat N, Demir O, Guven H, Esen A, Gidener S. Evaluation of vascular smooth muscle and corpus cavernosum on hypercholesterolemia. Is resveratrol promising on erectile dysfunction. Int J Impot Res 2010;22(4):227-33.
6. Gur S, Peak T, Kadowitz P, Sikka S, Hellstrom W. Review of erectile dysfunction in diabetic animal models. Curr Diabetes Rev 2014;10 (1):61-73.
7. Reiter RJ, Rosales-Corral S, Tan DX, Jou MJ, Galano A, Xu B. Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. Cell Mol Life Sci 2017;74:3863-81.
8. Cheng Y, Liu D zhou, Zhang C xiong, Cui H, Liu M, Zhang B le, et al. Mitochondria-targeted antioxidant delivery for precise treatment of myocardial ischemia–reperfusion injury through a multistage continuous targeted strategy. Nanomedicine 2019;16:236-49.
9. Czepas J, Koceva-Chyła A, Gwoździński K, Jóźwiak Z. Different effectiveness of piperidine nitroxides against oxidative stress induced by doxorubicin and hydrogen peroxide. Cell Biol Toxicol 2008;24(1):101-12.
10. Gioscia-Ryan RA, LaRocca TJ, Sindler AL, Zigler MC, Murphy MP, Seals DR. Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice. J Physiol 2014; 592(12):2549-61.
11. McCarthy C, Kenny LC. Therapeutically targeting mitochondrial redox signalling alleviates endothelial dysfunction in preeclampsia. Sci Rep 2016;6 :1-11.
12. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr Protoc 2021;1(4):e78.
13. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr Protoc Pharmacol 2015;70:1-21.
14. Wang Q, Zhang M, Torres G, Wu S, Ouyang C, Xie Z, et al. Metformin suppresses diabetes-accelerated atherosclerosis via the inhibition of Drp1-mediated mitochondrial fission. Diabetes 2017;66(1):193-

205.

15. Laight DW, Carrier MJ, Anggård EE. Antioxidants, diabetes and endothelial dysfunction. Cardiovasc Res 2000;47(3):457-64.
16. Sivitz WI, Yorek MA. Mitochondrial Dysfunction in Diabetes: From Molecular Mechanisms to Functional Significance and Therapeutic Opportunities. Antiox Redox Signal 2010;12(4):537-77.
17. Dikalova AE, Bikineyeva AT, Budzyn K, Nazarewicz RR, Lewis W, Harrison DG, et al. Therapeutic Targeting of Mitochondrial Superoxide in Hypertension. Circ Res 2010;107(1):106-16.
18. Dikalov SI, Dikalova AE. Contribution of mitochondrial oxidative stress to hypertension. Physiol Behav 2016;176(1):100-6.
19. Li X, Fang P, Li Y, Kuo Y-M, Andrews AJ, Nanayakkara G, et al. Mitochondrial Reactive Oxygen Species Mediate Lysophosphatidylcholine-induced Endothelial Cell Activation. Physiol Behav 2016;36(6):1090-100.
20. Ni R, Cao T, Xiong S, Ma J, Fan GC, Lacefield JC, Lu Y, et al. Therapeutic inhibition of mitochondrial reactive oxygen species with mito-TEMPO reduces diabetic cardiomyopathy. Free Radic Biol Med 2016;90:12-23.
21. Xing H, Zhang Z, Shi G, He Y, Song Y, Liu Y, Harrington EO, Sellke FW, ,et al. Chronic Inhibition of mROS Protects Against Coronary Endothelial Dysfunction in Mice with Diabetes. Front Cell Dev Biol 2021;9:643810.