Journal of Research in Pharmacy

Research Article

www.jrespharm.com

The study evaluating the effect of empagliflozin and dapagliflozin on miR-133a expression and oxidative stress in the rat heart induced by streptozotocin/nicotinamide

Humeysa KIYAK-KIRMACI [1,2,3], Ayse Nur HAZAR-YAVUZ [3], Elif Beyzanur POLAT [1,3], Hani ALSAADONI [4], Hanife Serife AKTAS [5], Hatice Kubra ELCIOGLU [3]*

1 Department of Pharmacology, Institute of Health Sciences, Marmara University, Kartal 34865 Istanbul, Türkiye.
2 Department of Pharmacology, Hamidiye Faculty of Pharmacy, University of Health Sciences, Uskudar 34668 Istanbul, Türkiye.
3 Department of Pharmacology, Faculty of Pharmacy, Marmara University, Maltepe 34854 Istanbul, Türkiye.
4 Department of Medical Biology, University of Health Sciences of Medicine, Uskudar 34668 Istanbul, Türkiye.
5 Department of Internal Medicine, Hamidiye Faculty of Medicine, University of Health Sciences, Uskudar 34668 Istanbul, Türkiye.
* Corresponding Author. E-mail: kubra.elcioglu@marmara.edu.tr (HK.E.).

Received: 26 April 2024 / Revised: 20 May 2024 / Accepted: 21 May 2024

ABSTRACT: Empagliflozin and dapagliflozin exert their effects by inhibiting sodium glucose cotransporter 2 (SGLT2), which inhibits glucose absorption from renal tubules. This class of drugs has also been demonstrated in studies to be protective against cardiovascular complications associated with type 2 diabetes mellitus (T2DM). Even in cases without T2DM, they have clinical utility due to their cardioprotective effects. The effects of empagliflozin and dapagliflozin on cardiovascular disorders remain incompletely understood. MicroRNAs (miRNAs) represent a class of small, non-coding RNA molecules that have been implicated in the pathogenesis of cardiovascular damage. miRNA expressions increase or decrease due to hyperglycemia and oxidative stress that occur in T2DM. This study intended to explore the SGLT2 inhibitor effects on miR-133a expressions in diabetic heart tissue by establishing a streptozotocin (STZ)/nicotinamide (NA)-induced diabetic rat model. Also, antioxidant activities were investigated in the heart and aorta tissue. Male-female Sprague-Dawley rats were injected with NA (100 mg/kg) and STZ (55 mg/kg) intraperitoneally (i.p.) respectively. One week after induction T2DM, treatments were carried out for four weeks. At the and of the treatment, the heart and thoracic aortic tissues of rats were removed. In the heart tissue glutathione (GSH), lipid peroxides (LPO), and myeloperoxidase (MPO) levels, and in the aorta tissue GSH and LPO levels were determined by fluorences method. miR-133a expression changes were assessed in the heart tissue by RT-PCR analyses. According to our results, dapagliflozin showed an antioxidant effect by increasing GSH levels in the heart ($p<0.01$) and aorta tissue more than empagliflozin. miR-133a expressions increased in the T2DM group and decreased in the EMPA ($p<0.05$) and DAPA groups ($p<0.01$). Studies on miR-133a expressions in different diabetes models are needed.

KEYWORDS: Type 2 Diabetes Mellitus; miR-133a; oxidative stress; cardiovascular complications; rat.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex public health problem. It is caused by impaired insulin secretion from pancreatic beta cells or resistance to insulin (IR) in the body [1]. The International Diabetes Federation (IDF) estimates that the number of people with DM will reach 643 million by 2045 [2]. Uncontrolled DM can lead to complications that affect various organs and may even cause death [3]. It mainly impairs endothelial function and raises mortality through cardiovascular complications [4]. Although control of hyperglycaemia is thought to be successful in preventing macrovascular complications, cardiovascular complications have recently become quite common even with tight glycaemic control. Therefore, alternative strategies are needed to prevent complications associated with DM [4, 5].

Hyperglycaemia causes oxidative stress and associated metabolic abnormalities in the diabetic heart disease. Oxidative stress occurs as a result of an imbalance between reactive oxygen species (ROS), including hydroxyl radical, NO, O2-, and hydrogen peroxide, and the antioxidant mechanism. Increased oxidative

How to cite this article: Kiyak-Kirmaci H, Hazar Yavuz AN, Polat EB, Alsaadoni H, Aktaş HŞ, Elçioğlu HK. The study evaluating the effect of empagliflozin and dapagliflozin on miR-133a expression and oxidative stress in the rat heart induced by streptozotocin/nicotinamide. J Res Pharm. 2024; 28(6): 1892-1900.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.862

stress causes tissue damage by triggering cells to undergo apoptosis [6]. Thus, hyperglycemia causes an increase in ROS in endothelial cells and myocytes, preventing the cell from suppressing oxidative stress [7]. The formation of free radicals can be reduced with targeted treatments that reduce ROS formation or increase antioxidants. Thus, vascular damage and cardiac dysfunction are reduced by preventing or regressing end-organ damage [8].

MicroRNAs (miRNAs) play a role in physiological and metabolic cellular signaling mechanisms including oxidative stress, which are small non-coding RNA molecules. Changes in the expression of miRNAs have been implicated in the pathogenesis of diseases including cancer, neurodegenerative diseases, heart disease, and DM [9, 10]. The crosstalk between miRNAs and oxidative stress is complex. miRNAs regulate genes related to oxidative stress. On the contrary, increasing amounts of ROS cause changes in miRNA expressions. The complex event between miRNA and ROS, especially in the heart tissue, has become very important in conditions such as diabetic cardiomyopathy, heart failure and cardiac hypertrophy [10].

miRNA expressions may change in pathogenesis as well as treatments and exposure to molecules that cause miRNAs to be upregulated or downregulated. Studies have shown that sodium-glucose cotransporter 2 (SGLT2) inhibitors modulate some miRNAs [11]. SGLT2 inhibitors act on the kidneys and ensure glucose excretion in the urine. Although it was developed as an oral antidiabetic drug, it is used in non-DM heart failure patients thanks to its cardioprotective effects [12, 13]. Empagliflozin reduces heart failure and cardiovascular mortality. In an animal model, empagliflozin has been shown to reduce oxidative stress, fibrosis, and macrophage infiltration in the myocardium [14]. Dapagliflozin reduces cardiovascular death and cardiac failure in patients with or without T2DM. Despite all this clinical data, the mechanism of the cardioprotective effects of SGLT2 inhibitors is still unclear [15].

Previous studies have shown that SGLT2 inhibitors have an antioxidant effect on heart tissue. However, the antioxidant effectiveness of empagliflozin and dapagliflozin, as SGLT2 inhibitors, has not been compared in studies. The study is the first to examine glutathione (GSH), lipid peroxides (LPO), and myeloperoxidase (MPO) levels in heart and aorta tissues in streptozotocin (STZ)/nicotinamide(NA)-induced T2DM rats treated with empagliflozin and dapagliflozin and to compare the effects of the two inhibitors. For this purpose, oxidative stress has been evaluated in heart and aorta tissues and then oxidative stress-related miRNA gene expression has been confirmed in heart tissue by RT-PCR analysis.

2. RESULTS

2.1. Results of blood glucose levels

The blood glucose levels of rats, which were monitored weekly during the experiment, are shown in Figure 1. During this time blood glucose levels in the T2DM rats were consistently higher than in the control group. At the end of 5 weeks, there was a significant increase in blood glucose levels in the T2DM group compared to the control group ($p<0.0001$). Throughout the trial, the rats receiving oral SGLT2 inhibitors had much lower blood glucose levels than the diabetic rats which had not taken treatment. Rats in the T2DM+D and T2DM+E groups had much lower blood glucose levels than those in the T2DM group ($p<0,0001$) at the end of the trial. Blood glucose levels were lower in the T2DM+D group than in the T2DM+E group (Figure 1).

2.2. Results of GSH, LPO and MPO levels

The GSH, LPO, and MPO levels of heart tissue are given in Figure 2. There are statistically significant differences between groups. The GSH level was decreased in the T2DM group compared to the control group ($p<0.05$). The GSH level was decreased in T2DM+E compared to the control group ($p<0.05$). GSH levels are significantly higher in the T2DM+D group compared to the T2DM ($p<0.05$) and T2DM+E ($p<0.01$) groups (Figure 2a). The LPO level was increased in T2DM group at a statistically significant rate compared to control in heart tissue ($p<0.5$). LPO levels were lower in the T2DM+E group compared to the T2DM group, and this difference was statistically significant ($p<0.05$). The LPO level of the T2DM+D group was higher compared to the T2DM+E group but did not show statistical difference with any group (Figure 2b). When MPO levels were examined in heart tissue in all groups, no significant difference was observed between the groups. However, unlike healthy animals, an increase in MPO levels was observed in all STZ/NA-induced groups (Figure 2c).

Journal of Research in Pharmacy
Research Article

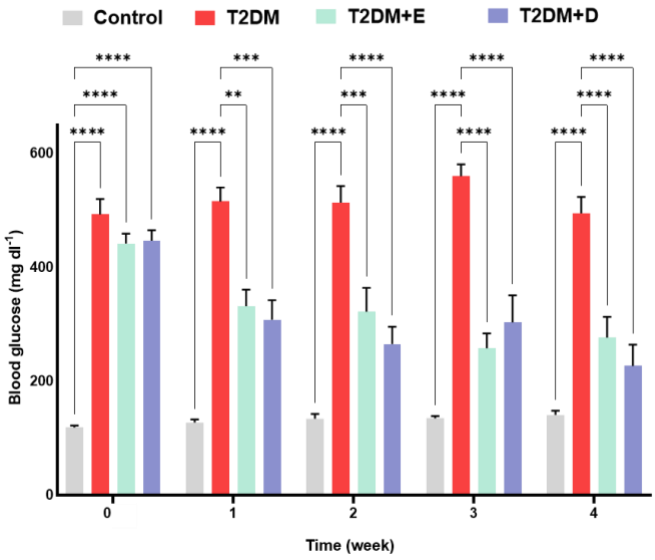

Figure 1. Blood glucose levels of control, T2DM, T2DM+E, and T2DM+D groups. T2DM: Type 2 diabetes mellitus; T2DM+E: Empagliflozin (10 mg/kg; p.o.) treatment administration group; T2DM+D: Dapagliflozin (1 mg/kg; p.o.) treatment administration group.

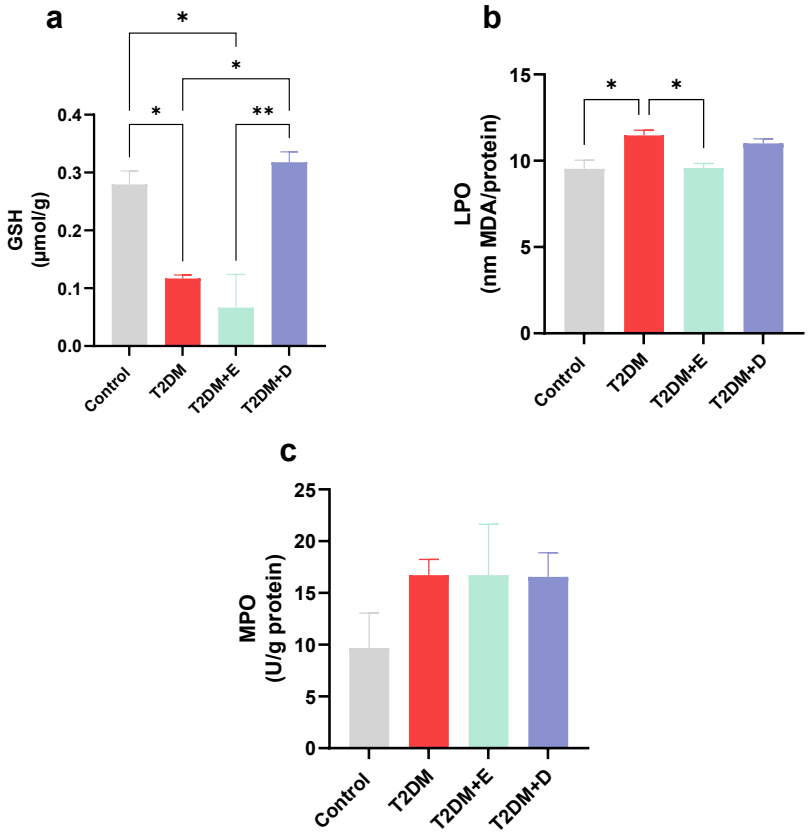

Figure 2. Result of a) GSH, b) LPO, and c) MPO levels in heart tissue of control, T2DM, T2DM+E, and T2DM+D groups. T2DM: Type 2 diabetes mellitus; T2DM+E: Empagliflozin (10 mg/kg; p.o.) treatment administration group; T2DM+D: Dapagliflozin (1 mg/kg; p.o.) treatment administration group.

The GSH and LPO results of aorta tissue are given in Figure 3. GSH levels were decreased in STZ/NA-induced animals compared to control. However, GSH levels did not show statistical significance

between the groups. Interestingly, the group with the lowest GSH levels was T2DM+E (Figure 3a). When the results of LPO levels were examined, no significant difference was shown between the groups in the aortic tissue. There was an increase in LPO levels in the T2DM group compared to the control and treatment groups, but this increase was not significant (Figure 3b).

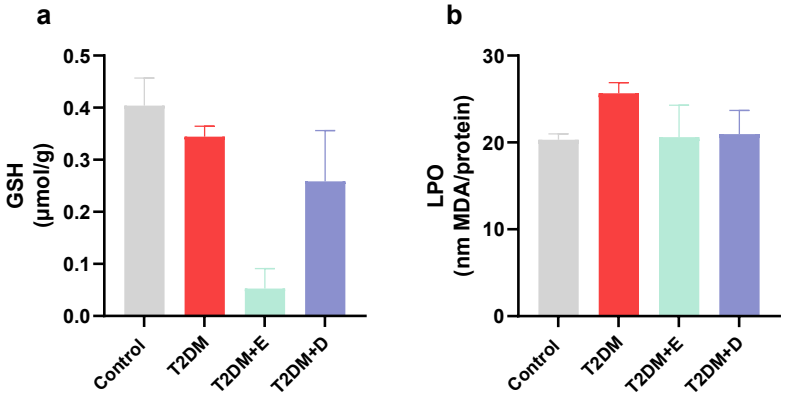

Figure 3. Result of a) GSH and b) LPO levels in aorta tissue of control, T2DM, T2DM+E, and T2DM+D groups. T2DM: Type 2 diabetes mellitus; T2DM+E: Empagliflozin (10 mg/kg; p.o.) treatment administration group; T2DM+D: Dapagliflozin (1 mg/kg; p.o.) treatment administration group.

2.3. Result of miR-133a analyses

The fold change results of miR-133a expression are given in Figure 4. There are statistically significant differences between groups. The miR-133a expression was decreased in the T2DM group compared to the control group ($p<0.05$). miR-133a expression was decreased in T2DM+E ($p<0.05$) and T2DM+D (0.05) compared to T2DM. There is no statistical difference between healthy rats and treated rat groups.

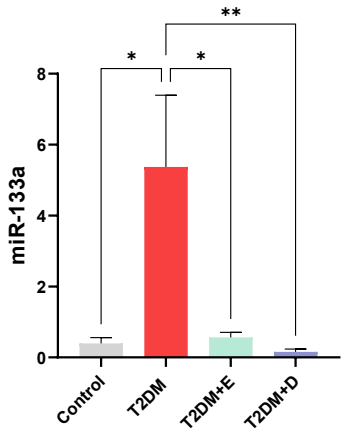

Figure 4. The 2-ΔΔCT fold change miR-133a results of control, T2DM, T2DM+E, and T2DM+D groups. T2DM: Type 2 diabetes mellitus; T2DM+E: Empagliflozin (10 mg/kg; p.o.) treatment administration group; T2DM+D: Dapagliflozin (1 mg/kg; p.o.) treatment administration group.

3. DISCUSSION

T2DM is associated with endothelial dysfunction, impaired vasodilation, increased oxidative stress, chronic inflammation, increased leukocyte adhesion, increased permeability and cellular degeneration [5]. The balance of ROS produced and accumulated due to hyperglycemia is disrupted, and the oxidant-antioxidant balances in the cells begin to change. For these reasons, scientists have focused on researching antioxidant substances and revealing the effects of some molecules. Molecules with antioxidant effects are a great advantage in preventing complications related to DM [16].

In this study, the antioxidant effects of the SGLT2 inhibitors empagliflozin and dapagliflozin were compared. Additionally, its effects on miR-133a, which is known to play a role in oxidation, were examined in heart tissue. In order to understand the effect of high glucose levels on oxidative parameters and miR-133a expression in heart tissue, T2DM was induced with STZ/NA in rats. Blood glucose was measured weekly and GSH, LPO, and MPO activities were evaluated in heart and aorta tissues. Finally, miR-133a expressions were confirmed by RT-PCR in heart tissue.

It was shown that the T2DM model was induced in rats 1 week after STZ/NA induction, depending on blood glucose levels. The blood glucose of all rats except the control group was above the reference ranges. Empagliflozin and dapagliflozin ensured that blood glucose levels in the treatment groups were significantly lower than those in the T2DM group throughout the study [17].

Hyperglycemia may increase oxidative stress and inflammation, which can lead to complications such as cardiomyopathy. Diabetic cardiomyopathy is usually asymptomatic until the disease progresses to advanced stages [18]. GSH as an antioxidant enzyme has a ubiquitous role. It has been shown that its deficiency increases structural changes in the vessels and myocardium [19].

Previous studies presented that GSH activity was decreased in T2DM hearts compared to healthy ones due to an impaired antioxidant defense system [20]. The findings of our study demonstrated that GSH activity in the heart decreases in the model of T2DM compared to other groups and were consistent with the literature. Studies have shown that dapagliflozin reduces tumor necrosis factor-α (TNF-α) induced ROS accumulation in endothelial cells compared to empagliflozin [12]. A more recent study has shown that empagliflozin protected endothelial permeability [12]. In our study, GSH activity was found to be higher in the T2DM+D group compared to the T2DM and T2DM+E groups. Although empagliflozin is used as a cardiac protector, in our results, GSH activity is quite low in the hearts of rats administered empagliflozin [21].

Similarly, in the aorta, GSH levels were found to decrease in diabetic groups, while LPO levels were found to increase. However, there were no statistically significant differences. The results of oxidant and antioxidant enzyme activities obtained in both heart and aortic tissue may be due to oxidative stress associated with hyperglycemia [22].

Uncontrolled overexpression of MPO is associated with adverse cardiovascular outcomes and an elevated risk of cardiovascular-related mortality [23]. MPO is an enzyme responsible for the oxidation of low-density lipoprotein (LDL) released by monocytic cells and neutrophils [24, 25]. MPO causes modifications in the oxidation of LDL and transforms to the atherogenic form. Additionally, it initiates lipid peroxidation [26]. According to our results MPO and LPO increased in T2DM group compared to control group in heart tissue. The MPO activity also increased in the treatment groups, which may be related to enhanced heart damage due to increased inflammation and oxidative stress. Unlike GSH, LPO activity was high in the dapagliflozin administered group and low in the empagliflozin group. Empagliflozin may have a protective effect at the stage where MPO induces LPO. The increase in LPO in the DM group is compatible with the literature [27].

Considering the effects of empagliflozin and dapagliflozin on miR-133a expression, there is a statistically significant increase in miR-133a expression in the T2DM group compared to all other groups. In contrast to the findings, previous studies showed that miR-133a expression was downregulated in the heart in the STZ-induced DM model. miR-133a plays a fundamental role in cardiac functions like fibrosis, hypertrophy, and cardiogenesis [28, 29]. Additionally, it was showed that an increase in miR-133a expression prevents fibrosis in the heart [30]. Deletion of the miR-133a gene causes irregular distribution, apoptosis, and proliferation patterns in cardiac muscles [28]. Unlike our study, mice were used in the heart injury models created in the literature. miR-133a expression has not yet been investigated in STZ-induced rat models [30, 31]. The reason for the results conflicting with the literature may be interspecies miRNA expression differences [32]. According to these results, there is a need to study miRNA expressions in more different DM models.

4. CONCLUSION

For the first time, we compared the antioxidant activities of SGLT2 inhibitors in STZ/NA-induced T2DM rat heart and aorta and examined their effects on miR-133a expression. Based on GSH activity results, dapagliflozin has more antioxidant effects on the heart and aorta than empagliflozin. However, neither drug completely could prevent the increase in LPO and MPO activity. Also, the effects of SGLT2 inhibitors on miR-133a expression should be supported by further studies.

5. MATERIALS AND METHODS

5.1. Experimental Design

Sprague-Dawley rats (250-350 g, n=32) were obtained from Marmara University Experimental Animals Application and Research Center. Rats were fed standard rat chow and ad libitum in a 12-hour light-12-hour dark room under constant humidity (50-60%) and temperature (22±2 °C) conditions. All the experimental protocols were approved by the Marmara University Animal Care and Use Committee (17.2024mar).

5.2. Induction of T2DM

After the one-week acclimation period is completed, the rats are randomly divided into 4 groups, with 8 rats in each group; control, T2DM, T2DM+E, and T2DM+D groups. The animals were administered freshly prepared STZ (55 mg/kg; i.p.) and NA (100 mg/kg; i.p.) in citrate buffer to induce T2DM. NA, which protects pancreatic cells, was administered 15 minutes before STZ. Treatments were started to be given intragastrically to the animals one-week after STZ administration. Rats with blood glucose above 200 mg/dl were considered as T2DM and included in the experiment. Only citrate buffer was administered to the control group. T2DM+E and T2DM+D groups were administered empagliflozin (10 mg/kg) and dapagliflozin (1 mg/kg), respectively. Citrate buffer, empagliflozin and dapagliflozin treatments were administered intragastrically for 28 days. The experimental timeline was showed in Figure 5.

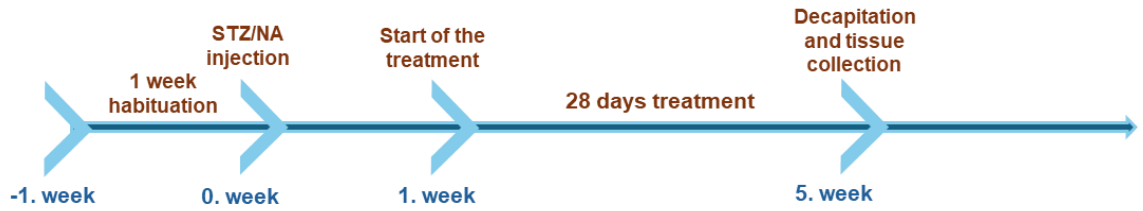

Figure 5. Experimental timeline.

5.3. Measuring of Blood Glucose Levels

Blood glucose measurement was made using a glucometer in the blood taken from the tail vein with an injector on the 0th, 7th, 14th, 21st, and 28th days in all experimental groups.

5.4. Sacrifice of Experimental Animals

At the end of 28 days of treatment, the animals were decapitated and the heart tissue and aorta were removed. After the left ventricle of the heart was removed, it was cut into small pieces and some of it was frozen in liquid nitrogen and stored at -80 °C and used for miRNA analysis. A part of the heart and aorta tissues were stored at -80 C to determine antioxidant activity.

5.5. Determination of LPO, GSH and MPO activities

Freshly thawed from −80°C heart (from 0.15 to 0.2 g) and aort (from 0.015 to 0.035 g) tissue samples were homogenized (Ultra Turrax homogenizer) in 10 volumes of ice-cold 10% trichloroacetic acid and centrifuged at 3000 rpm for 15 min at 4°C.

The levels MDA, a marker of lipid peroxidation, were measured in the heart and aorta tissues. MDA reacts with thiobarbituric acid (TBA) to form TBA reactive substances (TBARS), which produce a pink complex with a maximum absorbance at 532 nm. The supernatant was removed and recentrifuged at 15000 rpm for 15 minutes at 4°C. LPO levels were expressed as nmol MDA/g tissue, which is a measure of MDA equivalents.

GSH was determined using a spectrophotometric method, which is a modification of Ellman's method. After centrifugation, Na_2HPO_4-$2H_2O$ solution was added, and the absorbance at 412 nm was measured immediately after mixing. The results are expressed as mol GSH/g tissue.

Spectrophotometric testing was used to assess MPO enzyme activity as an indicator of polymorphonuclear leukocyte accumulation in inflamed tissues. The samples were homogenized in a cold potassium phosphate buffer (PBS, 20 mM K_2HPO_4, pH 7.4) and then centrifuged at 12,000 rpm for 10 minutes at 4°C. The resulting pellet was re-homogenized with a cold K_2HPO_4 solution containing 0.5% w/v HETAB. MPO activity was measured by assessing the H_2O_2-dependent oxidation of o-dianisidine 2HCl. Enzyme activity was determined at 460 nm and 37°C and expressed as a change in U/min [8].

5.6. miR-133a analyses

All reactions were carried out on ice. The left ventricles of the heart stored in RNA later were homogenized for miR-133a analysis. After homogenization, total RNA was extracted and the quality and purity of the RNA was determined by NanoDrop ND-100 spectrophotometer. Then, the RNA was reverse transcribed into single-stranded cDNA using miRCURY LNA RT Kit (Cat. No: 339340). U6 gene was used as the control gene. The sequence was created according to the sequences arriving at 100 ng purity. The accuracy of miR-133a expression was validated by miRCURY LNA SYBR Green PCR Kit (Cat. No: 339345). Reaction conditions were applied according to previous methods. The results obtained were measured with 2^ΔΔCT [33]. The U6 and miR-133a primer information is below (Table 1).

Table 1. miRNA primer sequence

miRNA	Forward sequence	Reverse sequence
U6	5′-CTCGCTTCGGCAGCACA-3′	5′-AACGCT TCACGAATTTGCGT-3′
rno-miR-133a	5′-TGCTTTGCTAGAGCTGGTAAAATG-3′	5′-AGCTACAGCTGGTTGAAGGG-3′

5.7. Statistical analysis

Statistical analyses were performed by Graph-Pad Prism 9.0 software. All of the results were expressed as mean ± standard error of the mean (SEM). One-way or two-way ANOVA was used followed by Tukey's test. The significance level adopted for all tests was value of $p < 0.05$.

Acknowledgements: This research was financially supported by the Marmara University Scientific Research Projects Unit (project number: TDK-2022-10415).

Author contributions: Concept – H.KK., AN.HY.; Design – H.KK., AN.HY., EB.P.; Supervision – HK.E.; Resources – AN.HY., H.A., HS.A.; Materials – HK.E., H.A., HS.A.; Data Collection and/or Processing – H.KK., AN.HY., EB.P.; Analysis and/or Interpretation – H.KK., EB.P.; Literature Search – H.KK., AN.HY., EB.P.; Writing – H.KK., AN.HY.; Critical Reviews – HK.E., AN.HY., H.A., HS.A.

Conflict of interest statement: The authors declare no conflict of interest for this publication.

REFERENCES

[1] Li M, Chi X, Wang Y, Setrerrahmane S, Xie W, Xu H. Trends in insulin resistance: insights into mechanisms and therapeutic strategy. Signal Transduct Target Ther. 2022;7(1):216. https://doi.org/10.1038/s41392-022-01073-0

[2] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119. doi: 10.1016/j.diabres.2021.109119. Erratum in: Diabetes Res Clin Pract. 2023;204:110945. https://doi.org/10.1016/j.diabres.2021.109119

[3] El-Shafey M, El-Agawy MSE, Eldosoky M, Ebrahim HA, Elsherbini DMA, El-Sherbiny M, Asseri SM, Elsherbiny NM. Role of Dapagliflozin and Liraglutide on Diabetes-Induced Cardiomyopathy in Rats: Implication of Oxidative Stress, Inflammation, and Apoptosis. Front Endocrinol (Lausanne). 2022 Mar;13:862394.https://doi.org/10.3389/fendo.2022.862394

[4] Guan H, Tian J, Wang Y, Niu P, Zhang Y, Zhang Y, Fang X, Miao R, Yin R, Tong X. Advances in secondary prevention mechanisms of macrovascular complications in type 2 diabetes mellitus patients: a comprehensive review. Eur J Med Res. 2024;29(1):152. https://doi.org/10.1186/s40001-024-01739-1

[5] Yu MG, Gordin D, Fu J, Park K, Li Q, King GL. Protective factors and the pathogenesis of complications in diabetes. Endocr Rev. 2024;45(2):227-252. https://doi.org/10.1210/endrev/bnad030

[6] Patel H, Chen J, Das KC, Kavdia M. Hyperglycemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. Cardiovasc Diabetol. 2013;12:142. https://doi.org/10.1186/1475-2840-12-142

[7] Knapp M, Tu X, Wu R. Vascular endothelial dysfunction, a major mediator in diabetic cardiomyopathy. Acta Pharmacol Sin. 2019;40(1):1-8. https://doi.org/10.1038/s41401-018-0042-6

[8] Arslan S, Ozcan O, Gurel-Gokmen B, Cevikelli-Yakut ZA, Saygı HI, Sen A, Goger F, Erkanli-Senturk G, Sener G, Tunali-Akbay T. Myrtle improves renovascular hypertension-induced oxidative damage in heart, kidney, and aortic tissue. Biologia. 2022;77(7):1877-1888. https://doi.org/10.1007/s11756-022-01039-1

[9] Shrivastav D, Singh DD. Emerging roles of microRNAs as diagnostics and potential therapeutic interest in type 2 diabetes mellitus. World J Clin Cases. 2024;12(3):525-537. https://doi.org/10.12998/wjcc.v12.i3.525

[10] Climent M, Viggiani G, Chen YW, Coulis G, Castaldi A. MicroRNA and ROS Crosstalk in cardiac and pulmonary diseases. Int J Mol Sci. 2020;21(12):4370. https://doi.org/10.3390/ijms21124370

[11] Shen Y, Cheng L, Xu M, Wang W, Wan Z, Xiong H, Guo W, Cai M, Xu F. SGLT2 inhibitor empagliflozin downregulates miRNA-34a-5p and targets GREM2 to inactivate hepatic stellate cells and ameliorate non-alcoholic fatty liver disease-associated fibrosis. Metabolism. 2023;146:155657. https://doi.org/10.1016/j.metabol.2023.155657

[12] Dyck JRB, Sossalla S, Hamdani N, Coronel R, Weber NC, Light PE, Zuurbier CJ. Cardiac mechanisms of the beneficial effects of SGLT2 inhibitors in heart failure: Evidence for potential off-target effects. J Mol Cell Cardiol. 2022;167:17-31. https://doi.org/10.1016/j.yjmcc.2022.03.005

[13] Hazar-Yavuz AN, Yildiz S, Keles Kaya R, Çam ME, Kabasakal L. Sodium-glucose co-transporter inhibitor dapagliflozin attenuates cognitive deficits in sporadic Alzheimer's rat model. J Res Pharm. 2022;26(2):298-310. https://doi.org/10.29228/jrp.128

[14] Furtado RHM, Bonaca MP, Raz I, Zelniker TA, Mosenzon O, Cahn A, Kuder J, Murphy SA, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Ruff CT, Nicolau JC, Gause-Nilsson IAM, Fredriksson M, Langkilde AM, Sabatine MS, Wiviott SD. Dapagliflozin and cardiovascular outcomes in patients with type 2 Diabetes Mellitus and previous myocardial infarction. Circulation. 2019;139(22):2516-2527. https://doi.org/10.1161/circulationaha.119.039996.

[15] Reid J, Rana K, Niman S, Sheikh-Ali M, Lewis T, Choksi RR, Goldfaden RF. Sodium-Glucose Cotransporter-2 (SGLT-2) Inhibitors for Cardiovascular Disease Prevention. Am J Cardiovasc Drugs. 2020;20(5):419-429. https://doi.org/10.1007/s40256-020-00397-y

[16] Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-1070. https://doi.org/10.1161/CIRCRESAHA.110.223545

[17] Alsereidi FR, Khashim Z, Marzook H, Gupta A, Al-Rawi AM, Ramadan MM, Saleh MA. Targeting inflammatory signaling pathways with SGLT2 inhibitors: Insights into cardiovascular health and cardiac cell improvement. Curr Probl Cardiol. 2024;49(5):102524. https://doi.org/10.1016/j.cpcardiol.2024.102524

[18] da Purificação NRC, Garcia VB, Frez FCV, Sehaber CC, Lima KRA, de Oliveira Lima MF, de Carvalho Vasconcelos R, de Araujo AA, de Araújo Júnior RF, Lacchini S, de Oliveira F, Perles JVCM, Zanoni JN, de Sousa Lopes MLD, Clebis NK. Combined use of systemic quercetin, glutamine and alpha-tocopherol attenuates myocardial fibrosis in diabetic rats. Biomed Pharmacother. 2022;151:113131. https://doi.org/10.1016/j.biopha.2022.113131

[19] Banerjee M, Vats P. Reactive metabolites and antioxidant gene polymorphisms in Type 2 diabetes mellitus. Redox Biol. 2014;2:170-177. https://doi.org/10.1016/j.redox.2013.12.001

[20] Verma S, Sagar N, Vats P, Shukla K, Abbas M, Banerjee M. Antioxidant enzyme levels as markers for type 2 diabetes mellitus. Int J Bioassays. 2013;2(4):685-690.

[21] Al Thani NA, Hasan M, Yalcin HC. Use of animal models for investigating cardioprotective roles of SGLT2 inhibitors. J Cardiovasc Transl Res. 2023;16(5):975-986. https://doi.org/10.1007/s12265-023-10379-5

[22] Ertik O, Tunali S, Acar ET, Bal-Demirci T, Ülküseven B, Yanardag R. Antioxidant activity and protective effects of an oxovanadium (IV) complex on heart and aorta injury of STZ-diabetic rats. Biol Trace Elem Res. 2024;202(5):2085-2099. https://doi.org/10.1007/s12011-023-03802-0
[23] Ndrepepa G. Myeloperoxidase - A bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta. 2019;493:36-51. https://doi.org/10.1016/j.cca.2019.02.022
[24] Osawa T. Development and application of oxidative stress biomarkers. Biosci Biotechnol Biochem. 2018;82(4):564-572. https://doi.org/10.1080/09168451.2017.1398068
[25] Calmarza P, Lapresta C, Martínez M, Lahoz R, Povar J. Utility of myeloperoxidase in the differential diagnosis of acute coronary syndrome. Arch Cardiol Mex 2018;88(5):391-396. https://doi.org/10.1016/j.acmx.2017.11.003
[26] Nicholls SJ, Hazen SL. The role of myeloperoxidase in the pathogenesis of coronary artery disease. Jpn J Infect Dis. 2004;57(5):S21-22.
[27] Kesavulu MM, Rao BK, Giri R, Vijaya J, Subramanyam G, Apparao C. Lipid peroxidation and antioxidant enzyme status in Type 2 diabetics with coronary heart disease. Diabetes Res Clin Pract. 2001;53(1):33-39. https://doi.org/10.1016/S0168-8227(01)00238-8
[28] Xiao Y, Zhao J, Tuazon JP, Borlongan CV, Yu G. MicroRNA-133a and myocardial infarction. Cell Transplant. 2019;28(7):831-838. https://doi.org/10.1177/0963689719843806
[29] Sang H-Q, Jiang Z-M, Zhao Q-P, Xin F. MicroRNA-133a improves the cardiac function and fibrosis through inhibiting Akt in heart failure rats. Biomed Pharmacother. 2015;71:185-189. https://doi.org/10.1016/j.biopha.2015.02.030
[30] Chen S, Puthanveetil P, Feng B, Matkovich SJ, Dorn GW, Chakrabarti S. Cardiac miR-133a overexpression prevents early cardiac fibrosis in diabetes. J Cell Mol Med. 2014;18(3):415-421. https://doi.org/10.1111/jcmm.12218
[31] Feng B, Chen S, George B, Feng Q, Chakrabarti S. miR133a regulates cardiomyocyte hypertrophy in diabetes. Diabetes Metab Res Rev. 2010;26(1):40-49. https://doi.org/10.1002/dmrr.1054
[32] Ha M, Pang M, Agarwal V, Chen ZJ. Interspecies regulation of microRNAs and their targets. Biochim Biophys Acta. 2008;1779(11):735-742. https://doi.org/10.1016/j.bbagrm.2008.03.004
[33] Li T-R, Jia Y-J, Wang Q, Shao X-Q, Zhang P, Lv R-J. Correlation between tumor necrosis factor alpha mRNA and microRNA-155 expression in rat models and patients with temporal lobe epilepsy. Brain Res. 2018;1700:56-65. https://doi.org/10.1016/j.brainres.2018.07.013