Ginger Extract and Omega-3 Fatty Acids Supplementation: A Promising Strategy to Improve Diabetic Cardiomyopathy

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Received October 17, 2023
Accepted March 12, 2024

Summary

Diabetic cardiomyopathy may result from the overproduction of ROS, TRPM2 and TRPV2. Moreover, the therapeutic role of ginger, omega-3 fatty acids, and their combinations on the expression of TRPM2 and TRPV2 and their relationship with apoptosis, inflammation, and oxidative damage in heart tissue of rats with type 2 diabetes have not yet been determined. Therefore, this study aimed to investigate the therapeutic effects of ginger and omega-3 fatty acids on diabetic cardiomyopathy by evaluating the cardiac gene expression of TRPM2 and TRPV2, oxidative damage, inflammation, and apoptosis in male rats. Ninety adult male Wistar rats were equally divided into nine control, diabetes, and treated diabetes groups. Ginger extract (100 mg/kg) and omega-3 fatty acids (50, 100, and 150 mg/kg) were orally administrated in diabetic rats for 6 weeks. Type 2 diabetes was induced by feeding a high-fat diet and a single dose of STZ (40 mg/kg). Glucose, cardiac troponin I (cTnI), lipid profile, insulin in serum, and TNF-α, IL-6, SOD, MDA, and CAT in the left ventricle of the heart were measured. The cardiac expression of TRPM2, TRPV2, NF-κB, Bcl2, Bax, Cas-3, and Nrf-2 genes was also measured in the left ventricle of the heart. An electrocardiogram (ECG) was continuously recorded to monitor arrhythmia at the end of the course. The serum levels of cTnI, glucose, insulin, and lipid profile, and the cardiac levels of MDA, IL-6, and TNF-α increased in the diabetic group compared to the control group ($p<0.05$). Moreover, the cardiac levels of SOD and CAT decreased in the diabetic group compared to the control group ($p<0.05$). The cardiac expression of TRPM2, TRPV2, NF-κB, Bax, and Cas-3 increased and Bcl2 and Nrf-2 expression decreased in the diabetic group compared to the control group ($p<0.05$). However, simultaneous and separate treatment with ginger extract and omega-3 fatty acids (50, 100, and 150 mg/kg) could significantly moderate these changes ($p<0.05$). The results also showed that the simultaneous treatment of ginger extract and different doses of omega-3 fatty acids have improved therapeutic effects than their individual treatments ($p<0.05$). It can be concluded that ginger and omega-3 fatty acids showed protective effects against diabetic cardiomyopathy by inhibiting inflammation, apoptosis and oxidative damage of the heart and reducing blood glucose and cardiac expression of TRPM2 and TRPV2. Combining ginger and omega-3 in the diet may provide a natural approach to reducing the risk or progression of diabetic cardiomyopathy while preserving heart structure and function.

Key words

Diabetic cardiomyopathy • Omega-3 Fatty acids • Ginger extract

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Introduction

Diabetic cardiomyopathy refers to a specific type of heart disease that occurs in individuals with diabetes, particularly those with poorly controlled blood glucose levels. It is characterized by structural and functional changes in the heart muscle that can lead to arrhythmia and heart failure [1]. The exact etiology of arrhythmia caused by diabetic cardiomyopathy is not fully understood, but several factors contribute to its

development. These include hyperglycemia, insulin resistance, oxidative stress, and inflammation [2]. High blood glucose levels in diabetes can lead to the accumulation of toxic byproducts such as advanced glycation end products (AGEs) and reactive oxygen species (ROS) [3,4]. These substances can cause damage to heart muscle cells and impair their function, and promote inflammation, fibrosis, and cell death [3,5]. Transient receptor potential melastatin 2 (TRPM2) and Transient receptor potential vanilloid channel 2 (TRPV2) are widely expressed in heart cells. Unlike voltage-gated ion channels, TRP channels do not have a typical voltage sensor but instead can sense a variety of other stimuli including pressure, shear stress, mechanical stretch, oxidative stress, lipid environment alterations, hypertrophic signals, and inflammation products. By integrating multiple stimuli and transducing their activity to downstream cellular signal pathways *via* Ca^{2+} entry and/or membrane depolarization, TRP channels play an essential role in regulating fundamental cell functions such as contraction, relaxation, proliferation, differentiation, and cell death [6,7]. TRPV2 is required for normal cardiac contractility. Overexpression of TRPM2 and TRPV2 impairs normal cardiac function and causes diastolic dysfunction [8,9]. Excessive expression of TRPV2 leads to chronic Ca^{2+} overload of cardiomyocytes, which may contribute to the development of cardiomyopathy [9]. TRPM2 activation induces cell injury and death by Ca^{2+} overload or enhanced inflammatory response [6]. Overall, the combination of these etiological factors and pathophysiological changes contributes to the development of diabetic cardiomyopathy, which can eventually lead to heart failure if left untreated or poorly managed. Although current treatment options focus on blood glucose control and management of cardiovascular risk factors, the use of multifaceted treatment strategies can play a prominent role in inhibiting the onset and progression of this disorder [10]. Emerging research suggests that natural compounds such as ginger and omega-3 fatty acids may have protective effects against diabetic cardiomyopathy [11,12].

Ginger (*Zingiber officinale*) is a widely used spice known for its anti-inflammatory, antioxidant, and anti-diabetic properties [11,13-16]. Several studies have demonstrated its potential protective effects against diabetic cardiomyopathy [11]. Ginger extract has been shown to reduce oxidative stress by increasing antioxidant enzyme activity and reducing lipid peroxidation in animal models of diabetes-induced cardiomyopathy [11,17]. Additionally, ginger's anti-inflammatory properties help suppress the release of pro-inflammatory cytokines, thereby reducing inflammation within cardiac tissues [18]. Furthermore, ginger has been found to improve glucose metabolism by enhancing insulin sensitivity and promoting glucose uptake in skeletal muscle cells [19]. By regulating blood glucose levels more effectively, ginger may indirectly protect against the development or progression of diabetic cardiomyopathy [20].

Omega-3 fatty acids are essential polyunsaturated fats found primarily in fish oil. Omega-3 fatty acids have been extensively studied for their potential protective effects against various cardiovascular diseases [12]. Omega-3 fatty acids have gained significant attention for their cardiovascular benefits due to their anti-inflammatory properties, triglyceride-lowering effects, and ability to improve endothelial function [21-23]. Studies have demonstrated that omega-3 supplementation can attenuate cardiac dysfunction by reducing oxidative stress markers [21]. Omega-3 fatty acids possess potent anti-inflammatory properties that can help mitigate the chronic low-grade inflammation associated with diabetes and its complications [22,24]. In diabetic cardiomyopathy, inflammation plays a crucial role in promoting fibrosis, oxidative stress, and apoptosis within the heart muscle. Omega-3 fatty acids have been shown to reduce pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), thereby attenuating inflammation and preventing cardiac damage [25]. Moreover, omega-3 fatty acids have been shown to improve insulin sensitivity, reduce blood glucose levels, and enhance lipid metabolism [26]. These metabolic benefits may contribute to the prevention or delay of diabetic cardiomyopathy development. It's important to note that more research is needed to fully understand the mechanisms underlying the protective effects of omega-3 on diabetic cardiomyopathy and to determine optimal dosages for therapeutic use. Hence, in the context of diabetic cardiomyopathy, omega-3 fatty acids have shown promising protective effects. However, the effect of treatment with omega-3 and ginger extract alone and in combination on the activity and expression of proteins TRPM2 and TRPV2 in the heart tissue of diabetic rats is not known. Therefore, the purpose of this study was to investigate the effect of 6 weeks of treatment with ginger extract, omega-3 and both together on diabetic cardiomyopathy in male rats by evaluating oxidative damage, inflammation, and the expression and activity of proteins TRPM2 and TRPV2 in the heart tissue.

Materials and Methods

Ginger extract preparation and its HPLC analysis

Fresh ginger or rhizome of *Zingiber officinale* Roscoe was purchased from a herbal shop and its botanical profile was confirmed by www.efloras.org and http://www.theplantlist.org. Preparation of ginger extraction was performed according to the method described by Akbari *et al.* [27]. High-Performance Liquid Chromatography (HPLC) analysis was performed to determine the levels of ginger bioactive compounds including, 6-gingerol, 8-gingerol, and 10-gingerol and 6-shogaol. The HPLC analysis protocol was based on the study of Ma and Li [16]. HPLC was performed by a device KNAUER equipped with a UV detector 2500 KNAUER set at 245 nm and consisted of a Smartline pump.

Animals and study design

Ninety adult male Wistar rats (weight: 260-300 g, age: 12-14 weeks) were equally divided into nine groups (n=10): control (C), type 2 diabetes (D), D-ginger extract (100 mg/kg) (DG100), D-omega-3 (50 mg/kg) (DOM50), DOM100 (100 mg/kg), DOM150 (150 mg/kg), DGOM50, DGOM100, and DGOM150. Type 2 diabetes was induced by consuming a high-fat diet (HFD) (a normal chaw with 10 % coconut oil and 5 % crude cholesterol) for three weeks along with administration of a dose of streptozotocin (40 mg/kg) as described previously [28] and fasting blood glucose level above 250 mg/dl were considered to diabetes [3]. Different doses of omega-3 fatty acids (50, 100, and 150 mg/kg) [29] and ginger extract (100 mg/kg) [27,30] were administered by oral gavage for six weeks. After the last treatment session, the animals were fasted overnight and then anesthetized by a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg). Animals were placed in a supine position on a temperature pad, body temperature was monitored with a rectal thermometer and maintained in the range of 37-38 °C. At the end of the course, after induction of anesthesia and before sampling, ECG was continuously recorded by a digital ECG recorder (SuzukenKenz, ECG 110, Suzuken Co., Japan). Blood samples were taken from the heart and its serum was separated by centrifuge (3000× g in 15 min) and stored at -20 °C. After that, the heart tissue was immediately separated, washed with ice saline and stored at -80 °C for molecular analysis.

Biochemical analysis

The serum level of glucose, total cholesterol (TC), triglycerides (TG), HDL, and LDL was measured using commercial kits. The serum levels of insulin and cardiac troponin I along with levels of TNF-α and IL-6 in the left ventricle of the heart were measured by ELISA method. The level of SOD was measured in the left ventricle of the heart using a commercial kit. CAT and MDA activity were estimated respectively according to the method of Aebi [31] and Buege [32] with some modifications in the left ventricle of the heart.

Real-time PCR

The gene expression of TRPM2, TRPV2, Bcl2, Bax, Caspase-3, NF-κB, and Nrf-2 (Table 1) was investigated in the left ventricle of the heart tissue. The Trizol method was used to isolate total RNA and a NanoDrop spectrophotometer device was used to evaluate RNA concentration. The synthesis of complementary (c)DNA was made by a Prime ScriptTM-RT reagent kit (TaKaRa). qRT-PCR was conducted in a real-time PCR system (RotorGene 6000, Corbett Research) using SYBR TM Green PCR Master Mix (Thermo Fisher Scientific). The internal control was β-actin and alterations in gene expressions were determined by $2^{-\Delta\Delta CT}$.

Histopathological examination

For histopathological evaluation, heart tissue was fixed in 10 % formaldehyde. Then tissue sections from the left ventricle of the heart with a thickness of 5 micrometers were prepared by a histologist and stained with hematoxylin. These sections were then examined by a light microscope (Nikon, Japan).

Statistical analysis

The results were statistically analyzed and displayed as means ± standard error of the mean (± SD). Statistical analysis was performed in the Statistical Package for Social Sciences (SPSS-19.0). The variables between groups were analyzed using a one-way analysis of variance (ANOVA). Where a significant difference was found with ANOVA, the source of difference was located followed by *post hoc* multiple comparisons and Tukey test for comparison. Statistical significance was set at $p<0.05$.

Results

HPLC

The Chromatogram obtained from HPLC analysis of ginger extract is presented in Figure 1. The results showed that the levels of 6-gingerol and 6-shaogol are at the highest

Table 1. Real-time PCR System Primer Sequences.

Gene name	**Primer sequences**	**Product length (bp)**
BAX	F5'-AGGGTGGCTGGGAAGGC-3' R5'-TGAGCGAGGCGGTGAGG-3'	93 bp
Bcl2	F5'-ATCGCTCTGTGGATGACTGAGTAC-3' R5'-AGAGACAGCCAGGAGAAATCAAAC-3'	134 bp
Caspase-3	F5'-GTGGAACTGACGATGATATGGC-3' R5'-CGCAAAGTGACTGGATGAACC-3'	135 bp
NF-κB	F5'-GCACCAAGACCGAAGCAAT-3' R5'-CGTAACCGCGTAGTCGAAGA-3'	143 bp
Nrf-2	F5'-AAAGACAAACATTCAAGCCGATTAG-3' R5'-TTGCTCCTTGGACATCATTTCAT-3'	141 bp
TRPV1	F5'-CAAGGCACTTGCTCCATTTG-3' R5'-TCTGTGGCCCAATTTCGA-3'	2721bp
TRPM2	F5'-GACAGCAACCACTCCCACTT-3' R5'-CTCCAACACCACGCAGACA-3'	162bp
β-actin	F5'-AAGATCCTGACCGAGCGTGG-3' R5'-CAGCACTGTGTTGGCATAGAGG-3'	327bp

peaks others. However, 8- and 10-gingerol were also detected by chromatograph in a smaller amount than others.

Treatment with omega-3 and ginger extract could improve body weight and heart weight in diabetic rats

The weight of body and heart tissue significantly increased in the diabetic group compared to the control group ($p<0.05$, Table 2). However, treatment with ginger extract and omega-3 fatty acids (100 and 150 mg/kg) could decrease body weight in the diabetic rats compared to the diabetic group ($p<0.05$, Table 2). The highest weight loss was observed in the DGOM150 group and the lowest weight loss was observed in the DOM50 group among all groups (Table 2).

Treatment with omega-3 and ginger extract could reduce the levels of glucose and insulin in serum and HOMA-IR in diabetic rats

Glucose and insulin levels were measured before and after diabetes induction to evaluate HOMA-IR levels. The results showed that the levels of these parameters before diabetes induction do not differ significantly between different groups. However, after the induction of diabetes, the levels of insulin and glucose showed a significant change and caused a significant increase in HOMA-IR compared to the control group and before induction ($p<0.05$, Table 3). Our results showed well that after the induction of diabetes, the levels of insulin, glucose, and HOMA-IR index significantly increased in the diabetic group compared to the control group ($p<0.05$, Table 3). However, treating with ginger extract (100 mg/kg), different doses of omega-3 fatty acids (50, 100, and 150 mg/kg), and ginger along with different doses of omega-3 could improve these parameters in rats with type 2 diabetes compared to the diabetic group ($p<0.05$). The results showed that glucose levels in the DGOM100 and DGOM150 groups were significantly reduced compared to the DG100, DOM50, DOM100, and DOM150 groups ($p<0.05$, Table 3).

Treatment with omega-3 and ginger extract could reduce the serum levels of lipid profile and glucose in diabetic rats

The results showed that HFD causes an increase in serum glucose, total cholesterol, LDL-c and triglyceride levels and a decrease in HDL-c levels in the diabetic group compared to the control group ($p<0.05$, Table 4). However, these parameters were reversely changed in the DG100, DOM50, DOM100, DOM150, DGOM50, DGOM100, and DGOM150 groups compared to the D group ($p<0.05$). The results showed that TG, TC, LDL-c, and HDL-c levels in the DGOM50, DGOM100, and DGOM150 groups were significantly reduced compared to the DOM50, DOM100, and DOM150 groups. The lowest level of TG, TC, and LDL-c and the highest level of HDL-c were observed in the DGOM150 group compared to other groups.

Treatment with omega-3 and ginger extract could reduce the serum levels of cardiac troponin I (cTnI) in diabetic rats

Serum levels of cardiac troponin increased significantly in the diabetic group compared to the control group ($p<0.05$, Fig. 2). However, its level in the DG100, DOM50, DOM100, DOM150, DGOM50, DGOM100, and DGOM150 groups was significantly reduced in comparison with the D group ($p<0.05$). Moreover, its levels in the DGOM50, DGOM100, and DGOM150 groups were significantly reduced compared to the DOM100 and DOM150 groups. The lowest level of cTnI was observed in the DGOM150 group compared to other groups.

Treatment with omega-3 and ginger extract could improve the levels of enzymes antioxidant and Nrf-2 expression in diabetic rats

The cardiac levels of SOD and CAT along with the cardiac expression of Nrf-2 decreased significantly and the MDA level increased in the diabetic group compared to the control group ($p<0.05$, Table 5). However, the activity of SOD and CAT increased and MDA level decreased in cardiac tissue of the DG100, DOM100, DOM150, DGOM50, DGOM100, and DGOM150 groups compared to the diabetic group ($p<0.05$, Table 5). Moreover, Nrf-2 expression in the DGOM50, DGOM100, and DGOM150 groups were significantly reduced compared to the DG100, DOM100, and DOM150 groups. The lowest level of MDA and the highest level of Nrf-2 and activity of SOD and CAT were observed in the DGOM150 group compared to other groups ($p<0.05$, Table 5 and Fig. 3).

Treatment with omega-3 and ginger extract could inhibit the levels of IL-6 and TNF-α and NF-κB expression in diabetic rats

The gene expression of NF-κB and the levels of IL-6 and TNF-α were significantly increased in the heart tissue in the diabetic group compared to the control group. However, the levels of these pro-inflammatory cytokines and the expression of this gene in heart tissue decreased in the DG100, DOM50, DOM100, DOM150, DGOM50, DGOM100, and DGOM150 groups compared to the diabetic group ($p<0.05$, Figs 4 and 5). The lowest expression of NF-κB and the levels of IL-6 and TNF-α were observed in the DGOM150 group compared to other groups ($p<0.05$, Figs 2 and 3).

Treatment with omega-3 and ginger extract could improve Bcl2 expression and inhibit Caspase-3 and Bax expression in diabetic rats

The cardiac expression of Bcl2 decreased and Cas-3 and Bax expression increased in the diabetic group compared to the control group ($p<0.05$, Fig. 6). However, their expression was inversely changed in the heart tissue in the DG100, DOM100, DOM150, DGOM50, DGOM100, and DGOM150 groups compared to the diabetic group. The results also showed that ginger alone can significantly change the expression of these genes in diabetic rats (DG100 group) compared to the diabetic group ($p<0.05$, Fig. 6). In addition, although the changes of these genes in the DOM50 group were not significant, a dose-dependent effect was observed for treatment with omega-3 in the DOM100 and DOM150 groups ($p<0.05$, Fig. 6).

Treatment with omega-3 and ginger extract could improve TRPM2 and TRPV2 expression in diabetic rats

The real-time PCR analysis showed that the gene expression of TRPM2 and TRPV2 in heart tissue increased significantly in the diabetic group compared to the control group ($p<0.05$, Fig. 7). However, their expression significantly decreased in the DG100, DOM100, DOM150, DGOM50, DGOM100, and DGOM150 groups compared to the diabetic group ($p<0.05$, Fig. 7). The lowest expression of TRPM2 and TRPV2 was observed in DGOM100 and DGOM150 groups compared to other groups ($p<0.05$, Fig. 7).

ECG findings

The findings of the electrocardiogram evaluation showed that the most arrhythmia observed in the diabetic group was sinus tachycardia (ST) (30 %, three out of ten rats), complete heart block (CHB) (20 %, two out of ten rats) and atrial fibrillation (AF) (10 %, and one rat out of ten rats). However, in the other groups, no disturbances in the electrical activity of the heart were observed by the monitoring of ECG (Fig. 8).

Histological findings

Histological examination of cardiac ventricular tissue in diabetic and healthy groups showed that induction of diabetes can dramatically change the structure of myocytes cause disorganization and induce hypertrophy in myocytes. It is well known that the existence of irregularity in the structure of myocytes and their irregular organization is one of the factors of disturbance in impulse transmission. However, these

changes showed significant improvement in diabetic groups treated with ginger extract and omega-3 fatty acids or both .Similar histological features were observed in diabetic rats treated with ginger extract, omega-3 and a combination of both (Fig. 9).

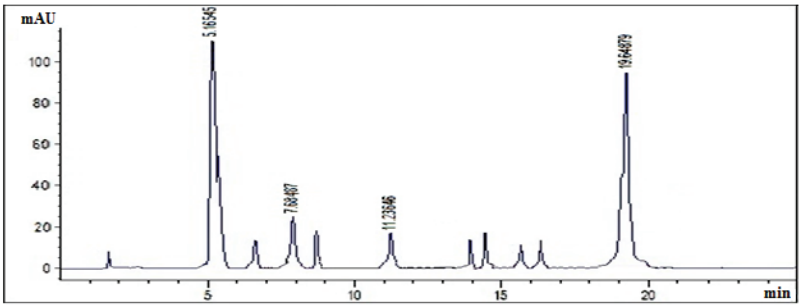

Fig. 1. Chromatogram obtained from HPLC analysis of ginger extract.

Table 2. The mean ± SD of body weight (g) and heart (g) in different groups.

Group	**Initial body weight (g)**	**Final body weight (g)**	**Heart weight (g)**	**HW/BW (%)**
Control	272.6±32.1	291.6±34.2	5.3±7.4	2.10
Diabetic	269.4±34.1	412.5±41.1**	8.4±3.4*	2.03
DG100	273.2±26.4	372.1±36.5$^{\#}$	6.1±3.1$^{\#}$	1.63
DOM50	267.1±25.1	390.4±35.4	6.2±4.2^{+}	1.54
DOM100	259.6±22.1	379.8±34.1^{+}	6.1±4.2^{+}	1.56
DOM150	274.7±20.7	367.4±34.1^{+}	6.2±6.2^{+}	1.68
DGOM50	274.2±22.4	345.2±27.4$^{\$}$	6.2±7.4$^{\$}$	1.79
DGOM100	271.2±20.4	319.4±28.4$^{\$\$}$	6.1±6.9$^{\$}$	1.90
DGOM150	266.9±21.4	284.4±29.1$^{\$\$}$	6.1±2.1$^{\$}$	2.22

The therapeutic role of ginger extract (100 mg/kg), Omega-3 fatty acids (50, 100, and 150 mg/kg), and their combinations on body weight and heart weight in the controlled and treated diabetic groups. DG100: Diabetes-Ginger 100. DOM50: Diabetes- Omega-3 fatty acids 50. DOM100: Diabetes- Omega-3 fatty acids 100. DOM150: Diabetes- Omega-3 fatty acids 150. * $p<0.05$ and ** $p<0.01$ vs. the control group. $^{\#}$ $p<0.05$ vs. the diabetic group. $^{+}$ $p<0.05$ vs. the diabetic group. $^{\$}$ $p<0.05$ and $^{\$\$}$ $p<0.01$ vs. the diabetic group.

Table 3. The mean ± SD of fasting blood glucose (mg/dl) and insulin (µU/l) in different groups.

	Fasting Blood Glucose (mg/dl)		**Insulin (µU/ml)**		**HOMA-IR**	
Group	**Before**	**After**	**Before**	**After**	**Before**	**After**
Control	91.2±8.4	95.4±9.7	10.2±1.1	11.4±1.4	1.32	1.49
Diabetic	93.4±7.8	362.2±21.2****	10.4±1.4	21.6±2.9*	1.36	6.8**
DG100	92.9±9.4	290.9±9.8$^{\#}$	11.2±1.1	14.3±2.1$^{\#}$	1.46	2.81$^{\#}$
DOM50	93.6±7.2	332.9±9.4^{+}	11.4±1.7	12.9±1.5^{+}	1.49	3.25^{+}
DOM100	91.2±2.2	317.9±2.4^{+}	11.2±0.7	13.1±0.5^{+}	1.45	2.99^{+}
DOM150	92.1±5.1	278.6±19.4^{++}	10.8±1.5	12.1±2.4^{+}	1.40	3.88
DGOM50	93.1±7.3	265.5±14.5$^{\$\$}$	11.1±2.1	16.4±1.4	1.45	3$
DGOM100	90.8±5.8	228.45±13.4$^{\$\$\$}$	11.3±1.8	12.1±2.4$^{\$}$	1.46	1.94$^{\$\$}$
DGOM150	90.1±4.1	168.45±10.4$^{\$\$\$\$}$	10.7±0.8	10.1±1.4$^{\$\$}$	1.32	1.5$^{\$\$}$

The therapeutic role of ginger extract (100 mg/kg), Omega-3 fatty acids (50, 100, and 150 mg/kg), and their combinations on serum levels of insulin and FBG in the controlled and treated diabetic groups. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$ vs. the control group. $^{\#}$ $p<0.05$ vs. the diabetic group. $^{+}$ $p<0.05$) and $^{++}$ $p<0.01$ vs. the diabetic group. $^{\$}$ $p<0.05$, $^{\$\$}$ $p<0.01$, $^{\$\$\$}$ $p<0.001$ and $^{\$\$\$\$}$ $p<0.0001$ vs. the diabetic group.

Table 4. The mean ± SD of lipid profile (mg/dl) and glucose (mg/dl) in different groups.

Group	TC (mg/dl)	TG (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)	Glucose (mg/dl)
Control	83.5±5.2	52.6±8.3	46.3±7.4	44.5±8.7	95.4±9.7
Diabetic	168.4±5.7**	87.4±4.1*	69.4±3.4*	18.5±2.1**	362.2±21.2***
DG100	138.7±6.4$^{\#}$	69.5±6.5$^{\#}$	53.1±3.1	25.1±2.3$^{\#}$	290.9±9.8$^{\#\#\#}$
DOM50	165.4±4.1	82.4±5.4	64.2±4.2	23.4±6.4^{+}	332.9±9.4^{+}
DOM100	159.48±6.3	78.48±4.1	61.1±4.2	26.1±2.4	317.9±2.4
DOM150	144.5±11.4^{+}	73.4±4.1^{+}	53.3±6.2^{+}	29.4±4.1^{+}	278.6±19.4^{+}
DGOM50	112.4±8.4$^{\$}$	66.5±7.4$^{\$}$	43.6±7.4$^{\$}$	36.4±2.1$^{\$}$	265.5±14.5$^{\$\$}$
DGOM100	99.4±10.5$^{\$\$}$	59.4±8.4$^{\$}$	39.4±6.9$^{\$}$	39.6±8.4$^{\$\$}$	228.45±13.4$^{\$\$\$}$
DGOM150	78.4±8.5$^{\$\$}$	51.1±9$^{\$\$}$	31.4±2.1$^{\$\$}$	47.6±3.2$^{\$\$\$}$	168.45±10.4$^{\$\$\$\$}$

The therapeutic role of ginger extract (100 mg/kg), Omega-3 fatty acids (50, 100, and 150 mg/kg), and their combinations on serum levels of lipid profiles and glucose in the controlled and treated diabetic groups. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes- Omega-3 fatty acids 100. DOM150: Diabetes- Omega-3 fatty acids 150. * p<0.05, ** p<0.01 and *** p<0.001 vs. the control group. $^{\#}$ p<0.05 and $^{\#\#\#}$ p<0.001 vs. the diabetic group. $^{+}$ p<0.05 vs. the diabetic group. $^{\$}$ p<0.05, $^{\$\$}$ p<0.01, $^{\$\$\$}$ p<0.001 and $^{\$\$\$\$\$}$ p<0.0001 vs. the diabetic group.

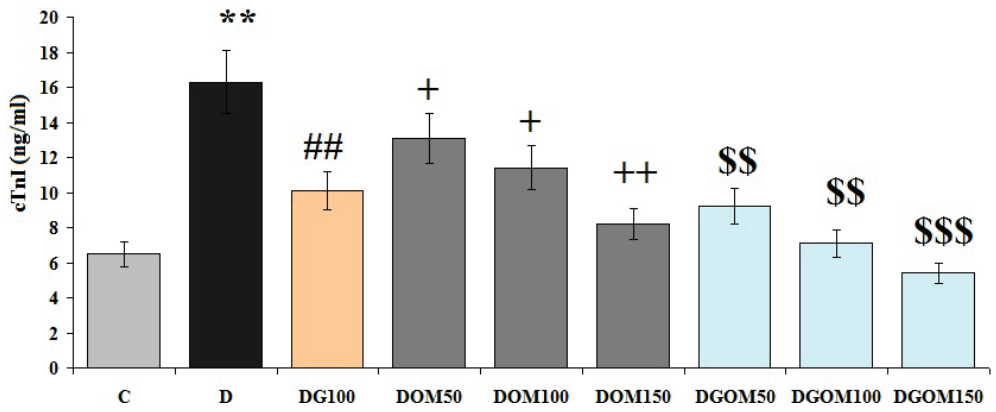

Fig. 2. The mean ± SD of cardiac troponin I (ng/ml) in different groups. * p<0.05 vs. the control group. ** p<0.01 vs. the control group. $^{+}$ p<0.05 vs. the diabetic group. $^{\#\#}$, $^{\$\$}$, and $^{++}$ p<0.01, and $^{\$\$\$}$ p<0.001 vs. the diabetic group. n=6. C: Control. D: Diabetes. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

Table 5. The mean ± SD of heart SOD, CAT, and MDA levels in different groups.

Group	SOD	CAT	MDA
Control	33.5±7.1	24.6±8.3	1.3±0.4
Diabetic	16.4±6.7**	11.4±4.1**	5.4±0.4***
DG100	29.7±6.4$^{\#\#}$	16.5±6.5$^{\#}$	3.1±0.1$^{\#\#}$
DOM50	17.4±4.1	12.4±5.4	4.6±0.2^{+}
DOM100	21.48±6.3	15.48±4.1	4.1±0.2^{+}
DOM150	25.5±11.4^{+}	17.4±4.1^{+}	3.3±0.2^{++}
DGOM50	28.4±8.4$^{\$}$	22.5±7.4$^{\$\$}$	2.6±0.4$^{\$\$}$
DGOM100	32.4±10.5$^{\$\$}$	24.4±8.4$^{\$\$}$	1.8±0.9$^{\$\$}$
DGOM150	36.4±8.5$^{\$\$}$	25.1±9$^{\$\$}$	1.1±0.1$^{\$\$\$}$

The therapeutic role of ginger extract (100 mg/kg), Omega-3 fatty acids (50, 100, and 150 mg/kg), and their combinations on the cardiac levels of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) in the controlled and treated diabetic groups. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150. ** p<0.01) and *** p<0.001 vs. the control group. $^{\#}$ p<0.05 and $^{\#\#}$ p<0.05 vs. the diabetic group. $^{+}$ p<0.05 and $^{++}$ p<0.01 vs. the diabetic group. $^{\$}$ p<0.05, $^{\$\$}$ p<0.01 and $^{\$\$\$}$ p<0.001 vs. the diabetic group.

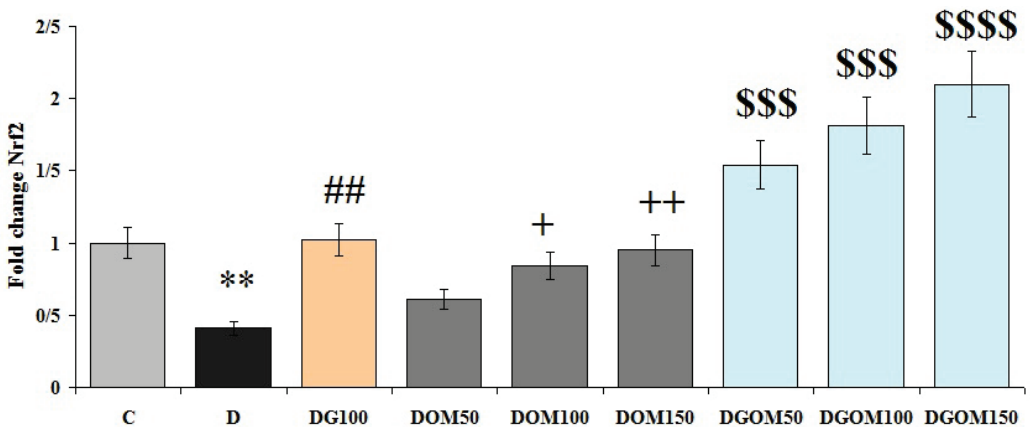

Fig. 3. The mean ± SD of cardiac expression of Nrf-2 in the controlled and treated groups. ** ($p<0.01$) vs. the diabetic group ** ($p<0.01$) vs. the control group. ## ($p<0.001$) vs. the diabetic group. + ($p<0.05$), ++ ($p<0.01$), $$$ ($p<0.001$), and $$$$ ($p<0.0001$) vs. the diabetic group. n=6. C: Control. D: Diabetes. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

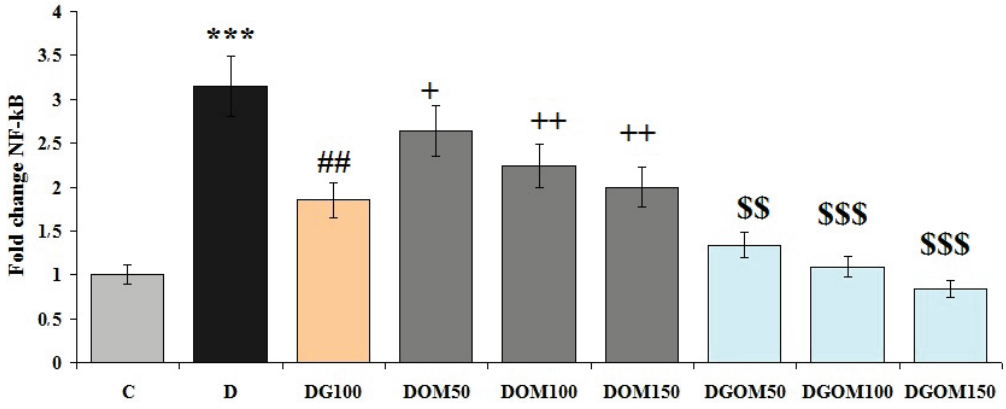

Fig. 4. The mean ± SD of the cardiac expression of NF-κB in the controlled and treated groups. *** ($p<0.001$) vs. the control group. ## ($p<0.001$) vs. the diabetic group. + ($p<0.05$), ##, $$, and ++ ($p<0.01$), $$$ ($p<0.001$) vs. the diabetic group. n=6. C: Control. D: Diabetes. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

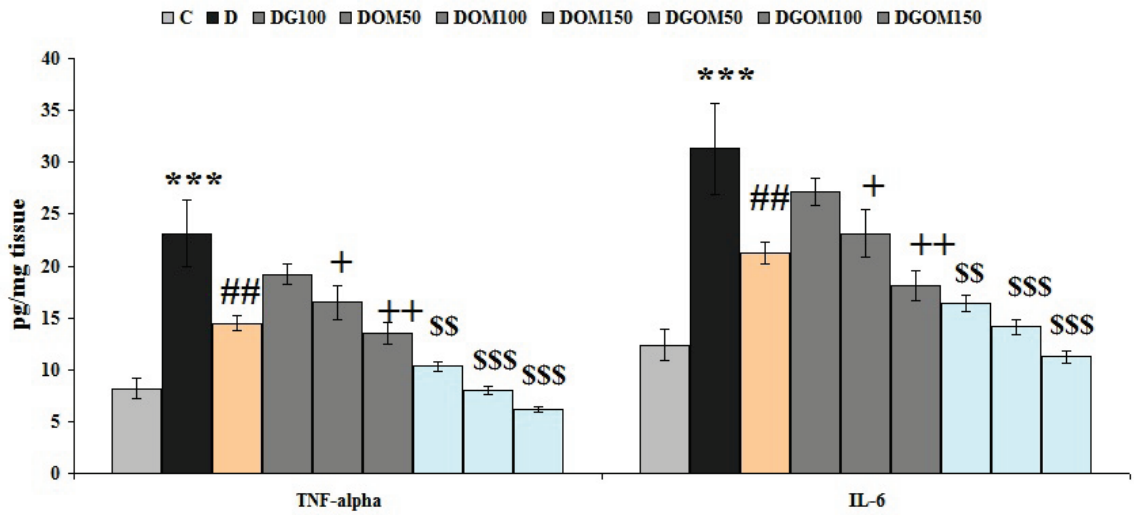

Fig. 5. The mean ± SD of the cardiac level of IL-6 and TNF-α in the controlled and treated groups. *** ($p<0.001$) vs. the control group. ## ($p<0.01$) vs. the diabetic group. + ($p<0.05$), ++ and $$ ($p<0.01$), $$$ ($p<0.001$) vs. the diabetic group. n=6. C: Control. D: Diabetes. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

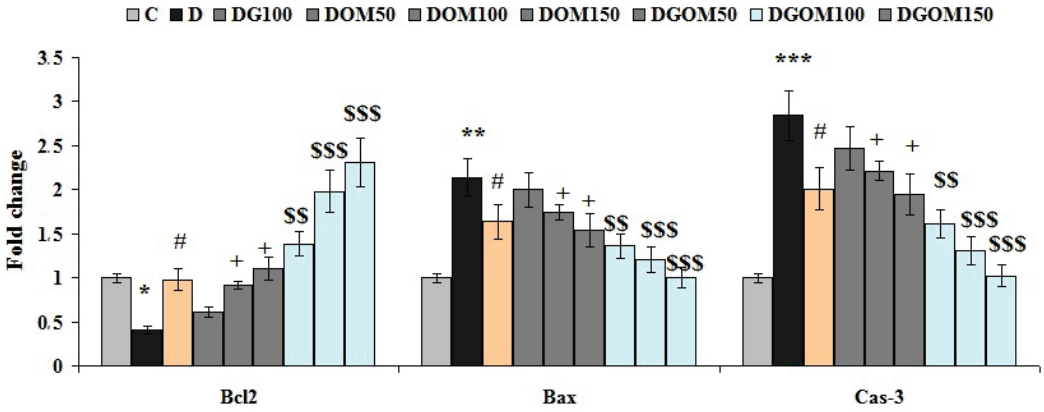

Fig. 6. The mean ± SD of the cardiac expression of Bcl2, Bax, and Caspase-3 in the controlled and treated groups. * (p<0.05), ** (p<0.01), and *** (p<0.001) vs. the control group. # (p<0.01) vs. the diabetic group. + (p<0.05), $$ (p<0.01), and $$$ (p<0.001) vs. the diabetic group. n=6. C: Control. D: Diabetes. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

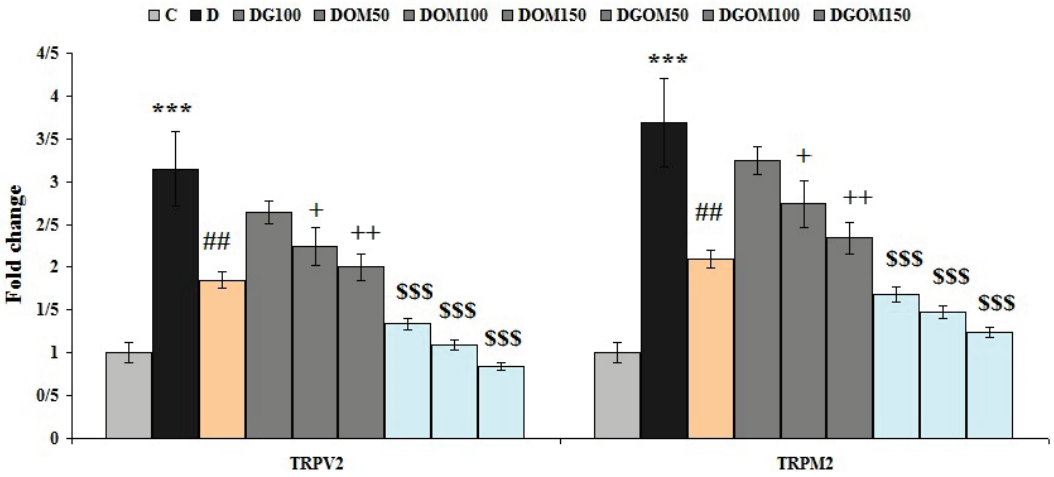

Fig. 7. The mean ± SD of the cardiac expression of TRPV2 and TRPM2 in the controlled and treated groups. *** (p<0.001) vs. the control group. + (p<0.05), ## and ++ (p<0.01) and $$$ (p<0.001) vs. the diabetic group. n=6. C: Control. D: Diabetes. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

Discussion

Diabetic cardiomyopathy is a serious complication of diabetes that affects the structure and function of the heart. The results of this study showed that diabetic cardiomyopathy is induced by inflammation, apoptosis, and oxidative damage. In addition, we have shown in this study that impairment of the activity and rate of TRPV2 and TRPM2 channels can play a role in the occurrence of diabetic cardiomyopathy. These factors, in addition to the disturbance in the structure of the heart, can be the cause of the disturbance in its electrical and dynamic activity. Serum levels of cardiac troponin I were increased in diabetic rats, which is a measure of cardiac damage. The histological results also showed the presence of inflammatory cells and changes in the structural characteristics of the heart in line with this evidence. Moreover, the results of the ECG evaluation also showed that there are arrhythmias such as ST, AF and CHB in diabetic rats. These results were in line with dyslipidemia and the increase of the HOMA-IR index, which clearly indicates a change in the metabolic state. However, treatment with ginger extract and different doses of omega-3 fatty acids or their simultaneous use can modulate these changes well and improve the effects of diabetes on heart structure and function.

Our results showed that the simultaneous treatment with ginger extract and omega-3 in different doses has a higher effect than the treatment with ginger alone or omega-3 alone in diabetic rats. In this study, we observed that the simultaneous use of ginger extract and different doses of omega-3 (50, 100, and 150 mg/kg)

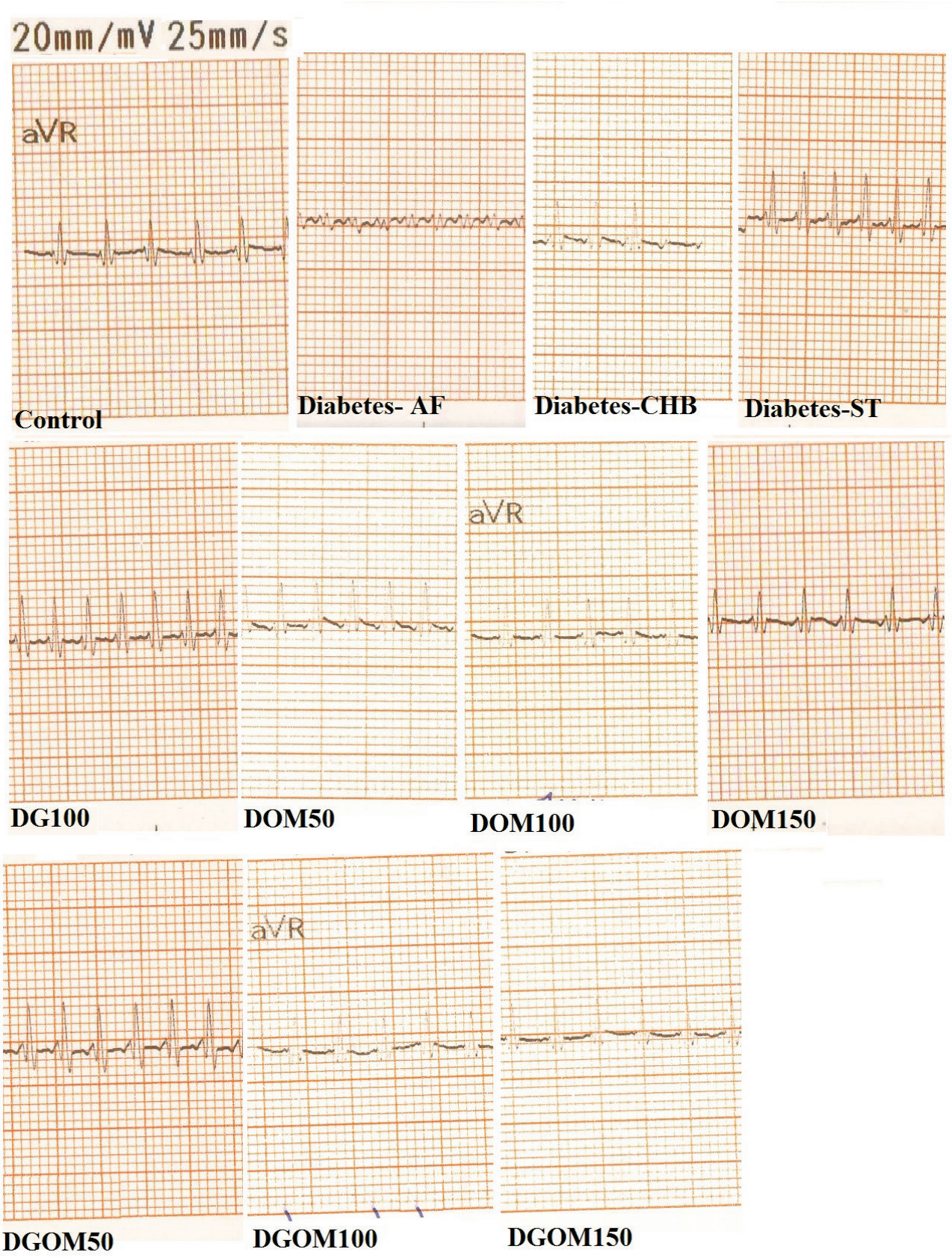

Fig. 8. A sample of ECGs taken at the end of the study period from control, diabetic and diabetic subjects treated with ginger, omega-3 and a combination of both. AF: Atrial fibrillation CHB: Complete Heart Block, ST: Sinus Tachycardia. DG100: Diabetes-Ginger 100. DOM50: Diabetes-Omega-3 fatty acids 50. DOM100: Diabetes-Omega-3 fatty acids 100. DOM150: Diabetes-Omega-3 fatty acids 150.

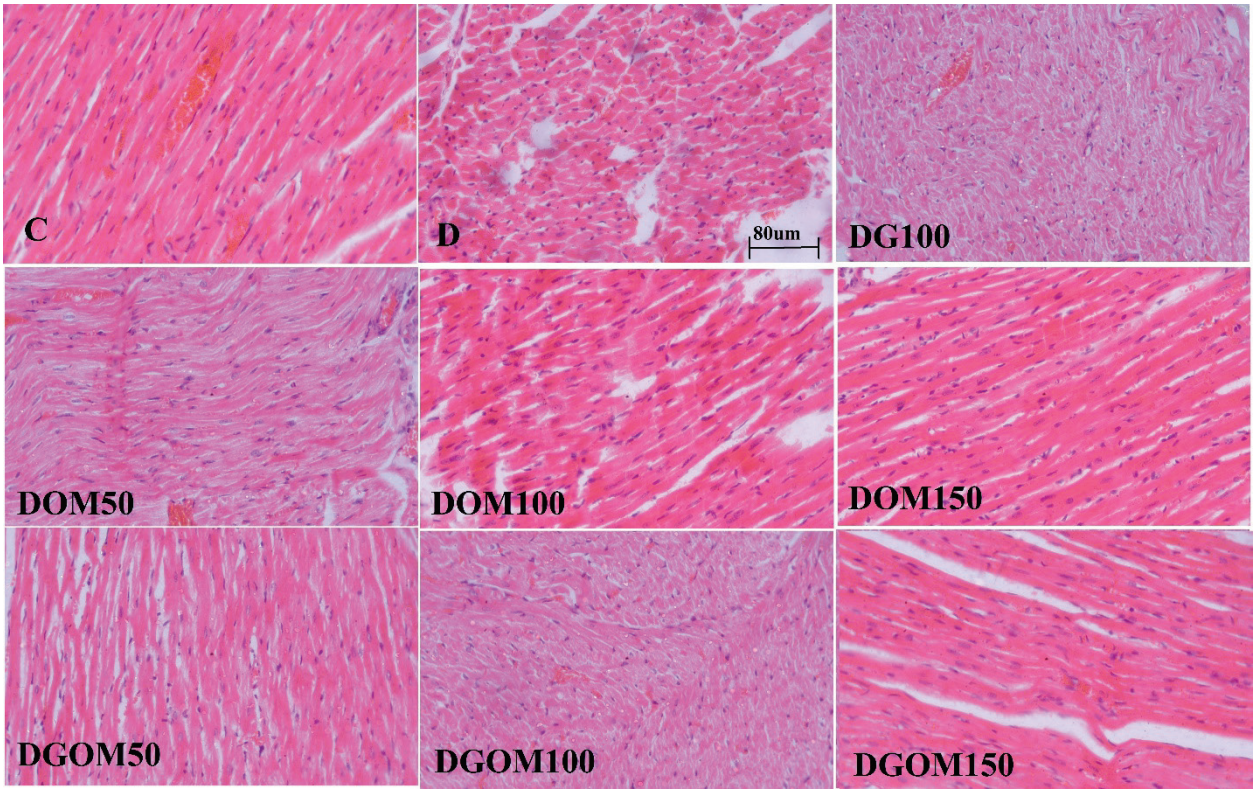

Fig. 9. The characteristics of diabetic cardiomyopathy in the cardiac tissue sections stained with hematoxylin and eosin of the left ventricle of the heart were examined under the light microscope with 40 magnifications in healthy, diabetic, and treated-diabetic groups. Cardiomyocyte hypertrophy and inflammatory cells are clearly visible in the diabetic group compared to the control group. However, these characteristics were not observed in the diabetic groups treated with ginger/omega-3 fatty acids compared to the diabetic group.

could improve the levels of serum factors including cTnI, lipid profile, insulin, and glucose. In the tissue of the heart, they also produce outstanding antioxidant, anti-apoptotic, and anti-inflammatory effects. Combined treatment of ginger and omega-3 fatty acids may offer synergistic effects in protecting against diabetic cardiomyopathy. Both compounds possess anti-inflammatory, anti-apoptotic, antioxidant, and lipid/glucose-regulating properties that can collectively target multiple pathways involved in the development and progression of diabetic cardiomyopathy. Furthermore, ginger's ability to enhance insulin sensitivity may complement omega-3's effects on glucose metabolism, leading to improved glycemic control. By reducing inflammation, oxidative stress, and apoptosis within cardiac tissues, this combination therapy holds promise for preventing or mitigating the detrimental effects of diabetic cardiomyopathy.

Abnormal lipid and glucose metabolism is a hallmark feature of diabetes, leading to hyperglycemia and dyslipidemia characterized by elevated triglycerides and reduced HDL-c levels. Ginger has been shown to have hypoglycemic effects by enhancing insulin sensitivity and glucose uptake in peripheral tissues [4,11]. By improving glycemic control, ginger may help prevent or delay the onset of diabetes-related complications such as cardiomyopathy. Ginger has been found to regulate lipid metabolism by reducing total cholesterol, triglycerides, and LDL-c levels while increasing HDL-c levels [11]. The exact mechanism of the protective effects of omega-3 fatty acids on diabetic cardiomyopathy is not fully understood, but several potential mechanisms, including anti-inflammatory, antioxidant, lipid-modulating, and anti-fibrotic properties have been proposed [33,34]. Omega-3 fatty acids may improve insulin sensitivity in individuals with diabetes [35]. Insulin resistance is a key factor in the development of diabetic cardiomyopathy, and by improving insulin sensitivity, omega-3 fatty acids may help protect against cardiac damage [26]. Omega-3 fatty acids have been also shown to lower triglyceride levels while increasing HDL cholesterol levels. This favorable lipid profile helps prevent the development and progression of diabetic cardiomyopathy by reducing lipid accumulation within the heart muscle.

The findings of the ECG evaluation showed that

the most arrhythmia observed in the diabetic group was ST (30 %, three out of ten rats), CHB (20 %, two out of ten rats) and AF (10 %, and one rat out of ten rats). However, in the other groups, no disturbances in the electrical activity of the heart were observed by the monitoring of ECG. Interpretation of these results is somewhat difficult and should be done with caution. It can be concluded from these results that firstly, only a certain type of arrhythmia was not observed in diabetic animals and secondly, arrhythmia was not observed in all diabetic rats. The latter can be due to the difference in severity of diabetes and cardiomyopathy in sick animals. The relationship between diabetes and arrhythmia is relatively complex and multifactorial and includes dysfunction in the autonomic nerves that control the electrical activity of the heart, dysfunction in the generation of action potentials, and its conduction in the atria and ventricles [36-38]. By carefully examining our results, we realize that the occurrence of various arrhythmias can be caused by disturbances in the initiation of electrical impulses and disturbances in their transmission pathways. Impaired impulse initiation can be due to increased spontaneous activity of cardiomyocytes (generating aberrant foci) or evoked activity that is expressed early or after delayed depolarization [39]. Conduction disorders that can cause reentry are caused by dysfunction in intercellular connexins and ion channels, structural remodeling of the myocardium (hypertrophy and/or fibrosis, obesity), and changes in refractory periods [40,41]. One of the damage hypotheses for the transmission path and foci of electrical activity in the heart is inflammation and oxidative damage resulting from hyperglycemia, which is well supported by our results and other studies [42-44]. Contrary to what we observed in diabetic rats with arrhythmia, no arrhythmia was observed in rats treated with different doses of omega-3 alone or simultaneously treated with ginger extract. This shows that treatment with omega-3 or ginger can modulate the activity of ion channels in heart cells. Oxidative stress and inflammation are key factors contributing to the development of diabetic cardiomyopathy and can be the main causes of arrhythmias in diabetes [45]. Increased levels of ROS due to decreasing antioxidant enzyme activity and Nrf-2 expression can damage cellular components and impair cardiac function. An abnormal increase in ROS levels activates calmodulin-dependent protein kinase II (CaMKII) and subsequently leads to hyperphosphorylation of RYR2 [46]. It causes arrhythmogenic leakage of calcium from the sarcoplasmic reticulum in ventricular and atrial myocytes [45-47]. This in turn triggers further calcium release that activates the Na^{+}/Ca^{2+} exchanger (NCX) and produces a transient inward sodium current that depolarizes the membrane [48]. It is well established that electrical dysfunction is coupled with contractile dysfunction in diabetes. Following diabetes, contractile dysfunction occurs with a change in the amount of NCX activity and the sensitivity of myofilaments to Ca^{2+} and a change in the amount of Ca^{2+} released from the endoplasmic reticulum [49,50]. Animal studies have shown that heart size, blood glucose and systolic blood pressure increase following diabetes. The contractile properties of ventricular myocytes including peak shortening (PS), time to peak shortening (TPS), time to 90 % retendering (TR90) and maximal shortening/ retending velocities (±dl/dt) are significantly altered following diabetes [50]. Moreover, many studies have shown that hyperglycemia-induced oxidative stress and inflammation play a role in the occurrence of diabetic arrhythmia [3,5]. In support of this evidence, the anti-oxidative and anti-inflammatory compounds may have anti-arrhythmic properties [45].

Our results also showed that the consumption of ginger and omega-3 fatty acids showed anti-inflammatory and antioxidant effects in the heart tissue. In a dose-dependent manner, omega-3 fatty acids could increase the expression of the Nrf-2 gene and the activity of SOD and CAT enzymes and strengthen the antioxidant system of the heart. Moreover, treating with different doses of omega-3 fatty acids alone or with ginger extract could downregulate cardiac NF-κB expression and the levels of TNF-alpha and IL-6. Treated diabetic groups with doses of 50, 100, and 150 mg/kg omega-3 fatty acids and ginger extract (100 mg/kg) could inhibit apoptosis by increasing Bcl2 expression and decreasing Bax and Cas-3 expression in heart tissue. Ginger contains bioactive compounds such as gingerols and shogaols that possess potent hypoglycemic and other pharmacological properties such as anti-inflammatory and antioxidative properties [16,30]. Ginger also contains various antioxidants such as zingerone and flavonoids that scavenge free radicals and reduce oxidative stress [51]. In line with this evidence, the results of our HPLC analysis also showed that ginger extract contains a very high content of 6-gingerol, 6-shaegol, 8- and 10-gingerol, which have strong antioxidant and anti-inflammatory effects [16]. These antioxidants also enhance endogenous antioxidant defense mechanisms within cells, protecting

against oxidative damage in the heart. These compounds inhibit the expression of NF-κB and the production of pro-inflammatory cytokines (TNF-α and IL-6), thereby reducing inflammation in cardiac tissues [15]. It is well known that inhibiting the expression of NF-κB and increasing the scavenging of free radicals causes apoptosis. By attenuating oxidative damage and inflammation, ginger may help prevent or slow down the progression of diabetic cardiomyopathy. Moreover, studies have demonstrated that ginger can protect against myocardial injury caused by various factors including ischemia-reperfusion injury, hypertension, and hyper-lipidemia – all conditions commonly associated with diabetic cardiomyopathy [11,52]. Ginger's protective effects are attributed to its ability to reduce oxidative stress, inflammation, and apoptosis in cardiac tissues. These mechanisms contribute to the preservation of cardiac structure and function. What we observed in the results of cardiac histology and biochemical analysis of serum cardiac troponin I in the diabetic groups treated with ginger extract.

Our results also showed that oral administration of omega-3 fatty acids in a dose-dependent manner could reduce serum glucose, insulin, HOMA-IR, lipid profile and cardiac troponin I levels in diabetic rats. In addition, it was able to significantly increase the expression of the Nrf-2 gene and the activity of antioxidant enzymes (SOD and CAT) and decrease MDA level in the heart tissue. Treatment with different doses of omega-3 also showed a significant anti-inflammatory role in the heart tissue by inhibiting the expression of NF-κB and reducing the levels of IL-6 and TNF-α in diabetic rats. Omega-3 fatty acids possess potent anti-inflammatory properties that can help mitigate the chronic low-grade inflammation associated with diabetes and its complications [53]. Inflammation plays a crucial role in promoting fibrosis, oxidative stress, and apoptosis within the heart muscle. Treating with doses of 50, 100, and 150 mg/kg omega-3 fatty acids could also inhibit apoptosis by increasing Bcl2 expression and decreasing Bax and Cas-3 expression in heart tissue. Omega-3 fatty acids have been shown to reduce the expression of NF-κB and the TNF-α and IL-6 levels [25], thereby attenuating apoptosis and preventing cardiac damage. Omega-3 fatty acids have been found to improve cardiac function in individuals with diabetic cardiomyopathy [53,54]. They enhance myocardial contractility, reduce left ventricular hypertrophy, and improve diastolic function [33,34]. These effects are attributed to their ability to modulate ion channels, enhance calcium handling, and improve energy metabolism within cardiac cells [7,21]. Omega-3 fatty acids have been shown to inhibit cardiac fibrosis by reducing transforming growth factor-beta (TGF-β) signaling and suppressing the activation of fibroblasts. By preventing cardiac fibrosis, omega-3 fatty acids help maintain normal cardiac structure and function. Moreover, omega-3 fatty acids consumption can change the lipid content of the cell membrane. Some lipid components of the cell membrane, such as arachidonic acid, in addition to inflammatory processes, as moderators of various biological functions, can affect the occurrence of arrhythmia [55,56]. Omega-3 fatty acids, alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) can modulate the lipid structure of the plasma membrane [57]. Omega-3 fatty acids have been shown to modulate ion channels involved in cardiac electrophysiology. By regulating membrane lipid content and ion channels, omega-3 fatty acids may help maintain normal heart rhythm and prevent arrhythmias associated with diabetic cardiomyopathy.

Our results showed that the expression and activity levels of TRPV2 and TRPM2 channels in heart tissue improve after treatment with different doses of omega-3 fatty acids. Omega-3 fatty acids and TRPV2 and TRPM2 channels have been implicated in the regulation of arrhythmia. It seems that omega-3 fatty acids can modulate the activity of these channels through different mechanisms. Omega-3 fatty acids, particularly EPA and DHA, are polyunsaturated fatty acids found in fish oil and certain plant sources. They have been extensively studied for their cardiovascular benefits, including their potential to reduce the risk of arrhythmias [57]. Omega-3 fatty acids have been shown to modulate various ion channels involved in cardiac electrical activity, including potassium channels, sodium channels, and calcium channels [58]. By affecting these ion channels, omega-3 fatty acids can influence the duration and amplitude of action potentials in cardiac cells, potentially reducing the occurrence of abnormal electrical impulses that can lead to arrhythmias. TRPV2 and TRPM2 channels are a type of ion channel found in sensory neurons and various other tissues, including the heart [9]. They are primarily known for their role in pain sensation and temperature regulation but emerging evidence suggests they may also play a role in cardiac electrophysiology [7]. Activation of these channels has been shown to modulate cardiac electrical activity by influencing action potential duration and refractoriness. Studies have suggested that TRPV2 and TRPM2 activation may promote arrhythmogenic effects

by increasing intracellular calcium levels or altering potassium channel function [59,60]. The present research has proposed a potential interplay between ginger or omega-3 fatty acids and TRPV2 and TRPM2 channels in regulating cardiac electrophysiology. It is suggested that omega-3 fatty acids or ginger inhibit TRPV2 and TRPM2 channel activity or downstream signaling pathways, thereby reducing the risk of arrhythmias associated with diabetes. Hence, incorporating omega-3-rich foods or ginger supplements into the diet may offer significant benefits for individuals with diabetes in terms of preserving cardiac structure and function. However, it is important to note that the exact mechanisms underlying the relationship between omega-3 fatty acids, ginger, TRPV2 and TRPM2 channels, and arrhythmia are still being investigated. Further research is needed to fully understand the complex interactions and potential therapeutic implications of these molecules in arrhythmia management.

Conclusions

Ginger and omega-3 fatty acids exhibit various protective effects against diabetic cardiomyopathy through their anti-inflammatory, anti-apoptotic, antioxidant, hypoglycemic, hypolipidemic, and cardioprotective properties. The bioactive compounds present in ginger contribute to these beneficial effects by reducing inflammation, oxidative stress, apoptosis, and improving glycemic control. Incorporating ginger as a natural supplement, omega-3, or both into the diet may offer a natural approach to mitigate the risk or progression of diabetic cardiomyopathy by preserving cardiac structure and function. However, further research is needed to determine optimal dosages and treatment durations for maximum efficacy in preventing or treating diabetic cardiomyopathy in humans before definitive recommendations can be made.

Conflict of Interest

There is no conflict of interest.

Abbreviations

AF, Atrial Fibrillation; AGEs, Advanced Glycation End Products; ALA, Alpha-Linolenic Acid; ANOVA, One-Way Analysis Of Variance; Bax, BCL2 associated X; Bcl2, B-cell lymphoma 2; CaMKII, Calmodulin-Dependent Protein Kinase II; Cas-3, Caspase-3; CAT, Catalase; CHB, Complete Heart Block; cDNA, Complementary DNA; cTnI, Cardiac Troponin I; DHA, Docosahexaenoic Acid; ECG, Electrocardiogram; ELISA, Enzyme-Linked Immunosorbent Assay; EPA, Eicosapentaenoic Acid; HDL, High-Density Lipoprotein; HFD, High-Fat Diet; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HPLC, High-Performance Liquid Chromatography; IL-6, Interleukin-6; LDL, Low-density lipoproteins; MDA, Malondialdehyde; NCX, Na^{+}/Ca^{2+} Exchanger; NF-κB, Nuclear factor kappa B; Nrf-2, Nuclear factor erythroid 2-related factor 2; PS, Peak Shortening; Real-time-PCR, Real-Time Polymerase Chain Reaction; RNA, Ribonucleic acid; ROS,: Reactive Oxygen Species; RYR2, Ryanodine Receptor 2; SD, Standard Deviation; SOD, Superoxide Dismutase; SPSS, Statistical Package for Social Sciences; ST, Sinus Tachycardia;STZ, Streptozotocin; TC, Total Cholesterol; TG, Triglycerides; TGF-β, Transforming Growth Factor-Beta; TNF-α, Tumor Necrosis Factor-Alpha; TPS, Time to Peak Shortening; TR90, Time to 90 % Retendering; TRP, Transient Receptor Potential; TRPM2, Transient Receptor Potential Melastatin 2; TRPV2, Transient Receptor Potential Vanilloid Channel 2

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