The effects of resveratrol and melatonin on cardiac dysfunction in diabetic elderly female rats

Received 08.11.2022

Accepted 12/1/2023

Nilufer Akgun-Unal[1], Serhan Ozyildirim[2], Omer Unal[3], Saltuk Bugra Baltaci[4], Rasim Mogulkoc[4], Abdulkerim Kasim Baltaci[4]

[1]Department of Biophysics, Medicine Faculty, Ondokuz Mayis University, Samsun, Turkey, [2]Department of Cardiology, Institution of Cardiology, Istanbul University-Cerrahpasa, Istanbul, Turkey, [3]Department of Physiology, Medical Faculty, Kirikkale University, Kirikkale, Turkey, [4]Department of Physiology, Medical Faculty, Selcuk University, Konya, Turkey

Summary

We aimed to investigate the effects of melatonin and resveratrol on diabetes-related papillary muscle dysfunction and structural heart disorders. The protective effect of resveratrol and melatonin supplementation on cardiac functions was investigated in a diabetic elderly female rat model. 16-month-old rats (n = 48) were allocated into 8 groups. Group1: Control, Group2: Resveratrol Control, Group3: Melatonin Control, Group4: Resveratrol and Melatonin Control, Group5: Diabetes, Group6: Diabetes Resveratrol, Group7: Diabetes Melatonin, Group8: Diabetes Resveratrol and Melatonin. Streptozotocin was injected intraperitoneally to the rats for experimental diabetes induction. Thereafter, resveratrol (intraperitoneal) and melatonin (subcutaneous) were administered for 4 weeks. Resveratrol and melatonin had a protective effect on the contractile parameters and structural properties of the papillary muscle, which was impaired by diabetes. it has been presented that diabetes impairs the contractile function of the papillary muscle for each stimulus frequency tested and the responses obtained as a result of Ca^{+2} uptake and release mechanisms from the Sarcoplasmic reticulum, and it has been observed that these effects are improved with resveratrol and melatonin injection. The decrease in myocardial papillary muscle strength in the diabetic elderly female rat can be reversed with the combination of resveratrol, melatonin and resveratrol+melatonin. Melatonin+resveratrol supplementation is no different from melatonin and/or resveratrol supplementation. Resveratrol and melatonin supplementation may have a protective effect on cardiac functions in a diabetic elderly female rat model.

Key words

Diabetes, Cardiac dysfunction, Melatonin, Resveratrol, ~~Rapillary~~ Papillary muscle.

Corresponding author

Nilufer Akgun-Unal, Department of Biophysics, Medicine Faculty, Ondokuz Mayis University, Samsun, Turkey.
e-mail: nilüfer.akgununal@omu.edu.tr

Short title

The effects of potential agents on diabetes-related papillary muscle dysfunction

Introduction

Diabetes mellitus (DM) is a global health problem that poses a significant threat to human health with its high morbidity and mortality rates [1]. It is estimated that approximately 380 million people worldwide have diabetes, and this figure will reach 439 million by 2030 [1]. DM results in various acute and chronic complications such as neuropathy, nephropathy, cardiomyopathy, microangiopathy, atherosclerosis, diabetic foot and retinopathy [2]. Diabetes-induced cardiovascular complications are the leading causes of mortality [3]. Therefore, it is crucial to determine an effective strategy to prevent cardiovascular complications in patients with diabetes [3]. The prevalence of DM rises to over 20% in the population over 60 years of age and its consequences worsen in the elderly population. It is estimated that approximately 25% of the elderly population has diabetes [4]. Moreover, the incidence of cardiovascular diseases in elderly diabetics is higher than in the non-diabetic elderly population [4]. Those with prediabetes with impaired fasting glucose and impaired glucose tolerance were considered a transitional period from normal glycemic metabolism to DM, demonstrating an increased risk for future progression of DM [4]. The “food” concept has significance beyond its function in regulating survival and nutrition in the prevention and treatment of disease states, [5]. Polyphenols are compounds that function in a wide range and are also synthesized by plants [5]. Interest in these compounds has risen in medical research recently. The most studied polyphenol in this group has been resveratrol (3,5,4,-trihydroxystilbene), which is predominantly found in grapes, red wine, and strawberries (grape and berry fruits, juicy and small grains) [6]. Along with its antioxidant capacity, favorable effects of resveratrol include regulation of ion channels and the activities and expression levels of proteins and enzymes associated with survival signals [7]. Studies on resveratrol have proved its promising effects on glucose metabolism [8]. Resveratrol increases insulin-dependent glucose uptake in skeletal muscle, hepatocytes and adipocytes with the activation of SIRT1 (silent information regulator 1) protein [9].

Besides its unique feature of regulating the circadian rhythm and sleep-wake cycle, melatonin has physiological effects as detoxification of free radicals, regulation of immune

functions, protection of nerve cells, anticancer effects, and improvement of cardiovascular functions, reproduction, and fetal development [10]. Melatonin acts on a broad spectrum of aging-related effects, including metabolic sensitivity, mitochondrial alteration, antioxidative protection of biomolecules and cellular infrastructures, sirtuin activation, and coordination of central and peripheral circadian regulators [11]. Moreover, melatonin has a strong potential as a treatment option despite its weak and uncommon toxicity profile [12]. Possible synergistic effects of melatonin and resveratrol are an intriguing research subject, owing to their similar beneficial properties which they execute by affecting different targets through different pathways, as in glucose and lipid metabolism [13]. Therefore, we planned the present study to analyze the effects of separate and combined resveratrol and melatonin supplementation on the mechanical and structural properties of the rat papillary muscle in an aged female diabetic rat model.

Material and Method

Experimental Animals and Groups

16-month-old female Wistar rats with an initial weight of 350-400 grams were used in all experiments. To minimize physiological variations, only female rats were preferred. This study protocol was approved by the Selcuk University Experimental Medicine Research and Application Center Experimental Animals Ethics Committee on December 12, 2018, with the decision number 2018-34.

The total number of experimental animals was 48, and they were randomly divided into 8 groups containing equal numbers of rats. Control Group (C) (n=6): The animals in this group were fed with standard rat chow. Resveratrol Control Group (CR) (n=6): The animals in this group fed with standard rat chow were additionally given resveratrol (5 mg/kg/day) for 4 weeks (i.p.). Melatonin Control Group (CM) (n=6): ~~The animals in this group fed with standard rat chow were additionally given subcutaneous melatonin (5 mg/kg/day) for 4 weeks.~~ Control rats in this group were administered subcutaneous melatonin (5 mg/kg/day) for 4 weeks. Resveratrol and Melatonin Control Group (CRM) (n=6): ~~In addition to standard rat feed, animals in this group were given i.p. for 4 weeks. Resveratrol (5mg/kg/day) and subcutaneous melatonin (5mg/kg/day) were administered~~. Control rats in this group were administered i.p. resveratrol (5 mg/kg/day) and subcutaneous melatonin (5 mg/kg/day) for 4 weeks. Diabetes Group (D) (n=6): Animals in this group, whose diabetes was induced by intraperitoneal administration of a single dose of streptozotocin (STZ) (40 mg/kg), were fed with standard rat chow. Diabetes Resveratrol Group (DR) (n=6): Resveratrol (5mg/kg/day) was administered i.p. for 4 weeks starting from the end of the seventh day following a single dose of STZ (40 mg/kg) injection.

Diabetes Melatonin Group (DM) (n=6): Diabetic rats in this group were administered subcutaneous melatonin (5 mg/kg/day) for 4 weeks, starting from the end of the seventh day following a single dose of STZ (40 mg/kg) injection. Diabetes Resveratrol and Melatonin Group (DRM) (n=6): Diabetic rats in this group were administered i.p. resveratrol (5 mg/kg/day) and subcutaneous melatonin (5 mg/kg/day) for 4 weeks starting from the end of the seventh day following a single dose of STZ (40 mg/kg) injection.

Throughout the injection, all animals were maintained at standard room temperature (21±1 ºC) and humidity at 12/12 light-dark cycles. 3 animals were housed per cage, and all animals were supplied with food and water ad libitum.

Experimental Applications

Experimental Diabetic Method

A single dose of 40 mg/kg intraperitoneal streptozotocin (STZ) "Sigma S-0130" dissolved in Sodium Citrate buffer was injected into rats in the diabetic groups (D, DR, DM, and DRM) to induce diabetes experimentally. Six days after injections, blood glucose levels were measured in the tail veins of the animals using a diagnostic glucose kit. Rats with blood glucose levels of 300 mg/dl and above were considered diabetic [14].

Resveratrol Application

After resveratrol (R5010-Sigma) was dissolved in ethanol, 5 mg/kg/day was administered intraperitoneally for four weeks to the rats forming the CR, CRM, DR and DRM groups.

Melatonin Administration

Commercially available melatonin (Sigma M-5250), dissolved in ethanol at a dose of 5 mg per kg of bodyweight of the experimental animal was injected subcutaneously at the same time (10 a.m.) every day for 4 weeks.

Papillary Muscle Isolation

Rats were anesthetized with i.p. 70 mg/kg ketamine (Richter Pharma AG, Australia) and 8 mg/kg xylazine (Bioveta PLC, Czech Republic). The hearts of the rats were quickly removed under general anesthesia and taken into the Krebs solution, the pH of which was adjusted to 7.40, and pre-gassed with a 95% O2 + 5% CO2 mixture. Then, they were taken into a petri dish, the bottom of which was covered with slygard gel, containing the same solution. The hearts were fixed from the right ventricle with the help of a small needle in this solution so that they could be seen from the dorsal region. An incision was made in the wall of the ventricle from

the level of the atrioventricular valve to the apex, and the ventricle was opened from this part and the papillary muscles were isolated with the help of microsurgical scissors, avoiding all kinds of pressure on the hearts.

Isometric Contraction Records and Protocols

Isolated papillary muscles were fastened at both ends with a 6/0 silk suture. Tissues were taken into an isolated organ bath (MAY IOBS99 Isolated Tissue Bath and Circulator, Commat Ltd.) with a volume of 30 ml containing fresh Krebs solution. The temperature of the Krebs solution was kept constant at 33 ºC by passing it through the heat jacket in the organ bath (MAY WBC 3044 Water Bath and Circulator, Commat Ltd.) [15]. One of the papillary muscles was connected to the micromanipulator and the other to a force transducer (FDT05 Force Displacement Transducer, Grass Co.), and the tension of the muscle was adjusted to see maximum contraction. Supramaximal square-shaped stimuli were given using a stimulator (MAY ISO 150-C Stimulus Isolation Power Supply). All contraction data were collected on the hard disk at a sampling rate of 1 kHz utilizing an analog-digital converter (MP36 Four-Channel Data Acquisition unit, Biopac System Inc.) and its software (BSL PRO 3.7.5, Biopac System Inc.).

After the papillary muscle was placed in the organ bath, square-shaped stimuli with a basic frequency of 0.2 Hz, 10-15V (supramaximal), 2 ms duration were given. Thereafter, to see the frequency-dependent contraction responses, square-shaped stimuli of 10-15V (supramaximal), and duration of 2 ms with 0.2; 0.5; 1; 2; 3; 4; 5 Hz frequencies were given respectively, and after 20 peak values were counted for each frequency, starting from the lowest frequency, the contraction curves were recorded while moving to a higher frequency [16].

In the other protocol, the responses to the anticipatory stimuli were recorded. To investigate the Ca^{2+} uptake-release mechanisms from the SR (Sarcoplasmic reticulum) 10, 20, 30, 40, 50, 60, 70-second waiting intervals were set between every 100-second recordings taken with 10-15 V, 2 ms duration square stimuli at 0.2 Hz frequency, and the parameters obtained by using this protocol were calculated from the first contraction curves recorded after the standby period [17]. Also the anesthetics used did not affect the results of the monitored parameters.

Examination of Histological Parameters

Tissues were taken into 10% formalin solution with a fixative/tissue ratio of 10/1. Each tissue was fixed at +4°C for at least 24 hours. It is embedded in paraffin. Sections with a thickness of 5 μm were taken on polylysine slides with a microtome. Hematoxylin-Eosin staining was done.

Data and Statistical Analysis

The parameters obtained from the contraction records were reported as contraction time (CT, ms) and contraction force (CF, $g.mg^{-1}$). The parameters (CT, CF) for each papillary muscle were obtained as the average of 10 data recorded at separate frequencies in the frequency-dependent contraction protocol, and the averages of the first contraction curves recorded at the end of each waiting period in the pre-wait stimulus protocol. All data were noted as mean ± standard error of mean (SEM) and normal distribution of data was tested with Kolmogorov - Smirnow. One-way ANOVA was used to compare the means of the data of the groups on contraction parameters from a single direction. In addition, the Tukey posthoc-test was used to determine between which groups the difference was. Differences at the $p<0.05$ level were considered significant.

Results

The blood glucose levels of all experimental groups are summarized in Table 1. It was observed that diabetes significantly increased the blood glucose levels compared to group C, CR, CM and CR+M ($p<0.05$).

Findings of Basic Contraction Parameters

The contraction recordings taken from the experimental groups with stimuli at a frequency of 0.2 Hz, which is considered as the basic parameter in myocardial papillary muscle, are shown in Fig.1.

~~**Fig. 1.**~~

Diabetes induced a significant decrease in CF parameters compared to the control group, while it caused a significant prolongation in the CT parameter ($p<0.05$). Among the treatment groups, while a significant difference was observed between the D group and DR, DM and DRM groups in the CF parameter, ~~a positive improvement was observed in the CT parameter, causing it to approach the control values.~~ indicates that a positive improvement in the CT parameter was found, but significance is not indicated.

Findings of Frequency Dependent Contraction Parameters

Diabetes caused a significant decrease in CF responses at all frequencies, compared to the control group (C) ($p<0.05$). Compared to the D group, the DR, DM, and DRM treatment groups displayed a favorable increase in the responses obtained with the 0.2; 0.5; 1; 2 and 3 Hz frequency stimuli and represented a significant protective effect against diabetes-induced CF deterioration. ($p<0.05$) (Fig. 2A, B, C). It was observed that diabetes significantly prolonged the CT obtained with all frequency stimuli compared to group C ($p<0.05$). A significant improvement was observed in the CT parameter in the treatment groups (Fig. 2D, E, F).

~~**Fig. 2.**~~

Findings of the Anticipatory Stimulus – Contraction Relationship

The mean CF values of the records taken with the relevant protocol are shown in Fig. 3A, B and C for each group. Although there was a significant decrease in CF values during the 70-second waiting period with diabetes compared to the C group ($p<0.05$), there was a favorable significant upswing in the CF values of ~~DR, DM and~~ DRM ~~treatment~~ groups when compared with the D group ($p<0.05$).

~~**Fig. 3.**~~

However, diabetes caused a prolongation in CT values for all periods in the recordings taken with pre-pending stimuli. Furthermore, when the DR, DM and DRM groups were compared with the C group, there was no significant difference during the 70-second waiting period ($p>0.05$) (Fig. 4A, B, C).

~~**Fig. 4.**~~

Histological Studies

All groups were evaluated in terms of cardiomyocyte morphology, deterioration in general architecture, expansion in extracellular space, and myocyte atrophy [18]. It was observed that cardiomyocyte morphologies and general architecture were preserved in groups C, CR, CM and CRM. However, in the diabetes group, deterioration in the morphology and general architecture of cardiomyocytes, increase in extracellular space, intracytoplasmic vacuoles, and appearance compatible with myocyte atrophy were noted. Compared to the D group, there was no significant difference between the DR and DM groups, but the general architecture and morphology were better in the DRM group (Fig. 5).

~~**Fig. 5.**~~

Discussion

Recovery of total mechanical activity of contractile cardiomyocytes deteriorated by streptozotocin-induced diabetes is more difficult or even impossible in old animals/humans compared to young individuals [19]. Diabetes-related changes in myocardial structure and function that are unassociated with coronary artery disease, arterial hypertension, heart valve disease, or congenital heart disease are defined as diabetic cardiomyopathy [20]. Various mechanisms play a role in diabetic cardiomyopathy, including metabolic changes, myocyte hypertrophy, myocardial interstitial fibrosis, apoptosis, microvascular disease, autonomic dysfunction, impaired energy production, changes in intracellular calcium homeostasis, and deterioration in myocardial contractile proteins [20]. For these reasons, it is known that contractile activities due to diabetes will be affected. It is known that the decrease in oxygen in the blood or excessive oxygen consumption of the muscle will cause fatigue and the contraction activities will be affected [21]. Similarly, high-frequency stimulation of the muscle produces muscle fatigue. Stimuli with a frequency of 0.2 Hz, which are known not to cause fatigue, were used to evaluate the basal parameters, and then contraction responses were recorded by increasing the stimulus frequency (0.5; 1; 2; 3; 4 and 5 Hz). Averages of parameters calculated from these contractions suggest that resveratrol and melatonin may have a protective effect against diabetes-impaired muscle function, shown in Fig. 1A, 1B, ~~2A, and 2B~~ 2A, 2B, 2C, 2D, 2E and 2F. Our findings regarding the myocardial papillary muscles of diabetic rats can be associated with the alterations in intracellular calcium homeostasis and dysfunction owing to the diminution in myocardial contractile proteins. Moreover, the systolic Ca^{2+} concentration can increase in direct proportion to the stimulus frequency [22]. This decrease in CF in the diabetes group can be attributed to two reasons. The first is the disruption of the calcium-dependent calcium release mechanism from the SR, which causes the increase in $[Ca^{2+}]_i$, and the latter is that the Ca^{2+} response of the myofilaments is affected.

Chen et al. also revealed that resveratrol treatment significantly improved cardiac hypertrophic remodeling and dysfunction due to pressure overload by mechanically inhibiting the immunoproteasome [23]. Therefore, they suggested that resveratrol, as a new immunoproteasome inhibitor, could be a therapeutic agent for the treatment of cardiac hypertrophy and dysfunction [23]. In our study, resveratrol supplementation improved the decreased CF responses of myocardial papillary muscles of diabetic rats to stimuli at different frequencies below 4 Hz (Fig. 2A) and this finding is in line with the report of Chen et al. which showed that resveratrol treatment significantly improved cardiac hypertrophic remodeling and dysfunction [23]. However, in our study, the decreased CF responses of myocardial papillary muscles of diabetic rats to stimuli at frequencies of 4 and 5 Hz were not affected by resveratrol supplementation (Fig. ~~2A~~ 2B). These findings of our study are consistent with the findings of a

previous study by Zhao et al. which concluded that the effects of resveratrol supplementation on guinea pig papillary muscles are time- and dose-dependent [24]. It has been reported that resveratrol improves muscle mass in the plantaris muscles of aged rats and that resveratrol supplementation significantly reduces the contraction force when compared with control groups at frequencies of 10, 20, and 50 Hz [25]. In our study, resveratrol supplementation reduced the CF responses of myocardial papillary muscles of control group rats to stimuli at all frequencies in the isolated organ bath. This finding was also compatible with the reports of ~~Brian~~ Bennett et al. [25].

Melatonin, a versatile molecule secreted rhythmically by the pineal gland, plays a cardiac protective role, especially in conditions such as ischemia-reperfusion injury, atherosclerosis, diabetic cardiomyopathy, pathological hypertrophy, and heart failure [26]. Melatonin can protect the diabetic myocardium from diastolic dysfunction [27]. Moreover, melatonin can alleviate cardiac fibrosis, by restraining extracellular matrix overaccumulation, which is noticed during the process of pathological fibrosis [28]. Therefore, there is substantial evidence that melatonin may play a potentially critical role in the treatment and prevention of fibrosis present in cardiac hypertrophy [29]. In our study, melatonin supplementation improved the decreased CF responses of myocardial papillary muscles of diabetic rats to stimuli up to 3 Hz frequency (Fig. ~~2A~~ 2B). This finding is consistent with the aforementioned reports, which state that melatonin supplementation may be effective in the treatment of restoring cardiac muscle functions. However, the decreased CF responses of diabetic rats to stimuli at frequencies of 4 and 5 Hz were not affected by melatonin supplementation in our study (Fig. ~~2A~~, 2B). This finding suggests that melatonin supplementation may have dose and time-dependent effects [30]. Another study investigating the effect of melatonin on blood flow in various vascular beds has concluded that exogenous melatonin alters vascular blood flow in humans [31]. In that study, arterial blood pressure and heart rate were measured in 10 healthy subjects in the supine position for 3 minutes, and it was found that melatonin did not change heart rate. However, renal blood flow rate and renal vascular conductivity were lower with melatonin supplementation compared to placebo [31]. In our study, melatonin supplementation reduced the CF responses of myocardial papillary muscles of control group rats to stimuli up to 4 Hz frequency in the isolated organ bath (Fig. 2A, 2B and 2C). This finding is also consistent with the aforementioned reports.

In the present study, combined administration of resveratrol and melatonin improved the reduced CF responses of myocardial papillary muscles of diabetic rats to stimuli at 0.2, 0.5, 1, 2, and 4 Hz frequencies in the isolated organ bath (Fig. 2A, 2B and 2C). We could not find a study in the literature in which resveratrol and melatonin were applied in combination on

myocardial papillary muscle CF and CT. However, a previous report presenting the cardioprotective effect of resveratrol+melatonin combination in an experimental myocardial infarction model indirectly supports our findings [32]. The modulating effect of both resveratrol and melatonin on myocardial papillary muscle contraction has already been discussed in the previous section [29]. To the best of our knowledge, our study is the first to report the effects of combined resveratrol and melatonin application on myocardial papillary muscle contraction.

Although frequency-contraction time measurements are not included much in the literature, in our study, when the stimulation at increased frequencies in diabetic rats was compared with the other groups, the prolonged CT values showed a tendency to improve with the combined supplementation of resveratrol, melatonin and resveratrol+melatonin (Fig ~~2B~~, 2D, 2E and 2F), but it was not statistically affected. This finding may suggest that the Ca^{2+} sensitivity of myofilaments may be reduced during the relaxation phase of the contractile responses.

Menadione causes a prominent augmentation in the force of contraction followed by irreversible contractures in isolated myocardial preparations. 2-methyl-1,4-naphthoquinone (menadione), its positive inotropic effect is related to the amount of ROS produced by cardiac metabolism. Resveratrol supplementation improves these adverse events and leads to a cardioprotective effect [33]. Deterioration in isometric contraction was detected in rats due to melatonin deficiency resulting from pinealectomy [34]. Numerous studies have revealed that melatonin supplementation can modulate impaired heart muscle functions [29, 30]. Studies have shown that prolonging the rest period between stimuli will increase the force of contraction [35, 36]. In our study, a post-rest augmentation protocol was applied to investigate the mechanisms that release Ca^{2+} uptake from the SR in isolated rat papillary muscle. For this purpose, 10-minute increment periods were expected between pulse sequences and the data obtained are shown in Fig. ~~3~~ 3A, 3B and 3C. The parameters investigated in this protocol were calculated from the first contraction curve after the rest period [17]. According to these calculations, diabetes significantly reduced its contractile strength up to 70 seconds, and after 60 seconds, resveratrol, melatonin and resveratrol+melatonin application started to reduce its effect. This finding shows that the damage expected to occur in SR with this application can be reversed by waiting. Dysfunction of SR-Ca^{+2} load mechanisms in the diabetes group may compensate for waiting times beyond 70 seconds. However, supplementation of resveratrol, melatonin, and combined resveratrol+melatonin did not cause any alteration in the prolonged CT values of diabetic rats during the 70-second (10-minute intervals) waiting period in our study (Fig.~~4~~ 4A, 4B and 4C). These findings may be due to the fact that diabetic rats consisted of elderly female rats and/or the dose and duration of administration.

In our study, we performed histological examinations to investigate whether the underlying cause of the contractile defects observed in myocardial papillary muscles was diabetes, and when histological parameters were examined, diabetes-related abnormalities were detected. Impaired cardiomyocyte morphology and general architecture, increased extracellular space, intracytoplasmic vacuoles, and myocyte atrophy were regarded as histological evidence of cardiac papillary muscle dysfunction in the diabetes group (Fig. 5). These findings were more likely towards cardiac atrophy with reduction in cardiomyocytes, features of STZ-induced diabetic cardiac dysfunction. These findings were also reported in a previous study [37]. The results of the current study showed that treatment with melatonin and resveratrol reduced cardiac damage in STZ-induced diabetic rats.

Conclusion

Cardiovascular complications associated with diabetes are substantial causes of mortality, especially in the elderly. Therefore, studies on this subject can directly contribute to human health. Melatonin and resveratrol, which are effective agents to prevent cardiovascular complications in diabetic patients, have the potential to prevent dysfunction in the cardiac muscle. The degree of these changes in contraction parameters may be the subject of additional research, due to the effects of mechanisms such as SR/myofibril damage, administered dose and duration.

Acknowledgements

Conflict of interest The authors declare that they have no potential conficts of interest to disclose.

Ethical approval This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Selcuk University's Experimental Medicine Research and Application Center (2018-34). This research was performed on the animals (rat).

Funding This study was supported by the Selcuk University Faculty Member Training Program Coordinator (2016-OYP-091).

References

1. Nentwich MM, Ulbig MW. Diabetic retinopathy ocular complications of diabetes mellitus. World J Diabetes 2015; 6(3):489-499.
2. Changrani NR, Chonkar A, Adeghate E, Singh J. Effects of streptozotocin-induced type 1 diabetes mellitus on total protein concentrations and cation contents in the isolated pancreas, parotid, submandibular, and lacrimal glands of rats. Ann N Y Acad Sci 2006;1084:503-519.

3. Gili M, Orsello A, Gallo S, Brizzi MF. Diabetes-associated macrovascular complications: cell-based therapy a new tool? Endocrine 2013; 44(3):557-575.
4. Sonne DP, Hemmingsen B. Comment on American Diabetes Association. Standards of Medical Care in Diabetes. Diabetes Care 2017;40:1-135.
5. Dolinsky VW, Dyck JR. Experimental studies of the molecular pathways regulated by exercise and resveratrol in heart, skeletal muscle and the vasculature. Molecules 2014; 19(9):14919-14947.
6. Raj P, Louis XL, Thandapilly SJ, Movahed A, Zieroth S, Netticadan T. Potential of resveratrol in the treatment of heart failure. Life Sci 2014; 95(2):63-71.
7. Kwon KJ, Kim HJ, Shin CY, Han SH. Melatonin Potentiates the Neuroprotective Properties of Resveratrol Against Beta-Amyloid-Induced Neurodegeneration by Modulating AMP-Activated Protein Kinase Pathways. J Clin Neurol 2010; 6(3):127-137.
8. Pollack RM, Crandall JP. Resveratrol: therapeutic potential for improving cardiometabolic health. Am J Hypertens 2013; 26(11):1260-1268.
9. Breen DM, Sanli T, Giacca A, Tsiani E. Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. Biochem Biophys Res Commun 2008; 374(1):117-122.
10. Samanta S. Physiological and pharmacological perspectives of melatonin. Arch Physiol Biochem 2020:1-22.
11. Hardeland R. Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms. J Pineal Res 2013; 55(4):325-356.
12. Reiter RJ, Pablos MI, Agapito TT, Guerrero JM. Melatonin in the context of the free radical theory of aging. Ann N Y Acad Sci 1996; 786:362-378.
13. Majumdar AS, Giri PR, Pai SA. Resveratrol- and melatonin-abated ovariectomy and fructose diet-induced obesity and metabolic alterations in female rats. Menopause 2014; 21(8):876-885.
14. Havel PJ, Uriu-Hare JY, Liu T, Stanhope KL, Stern JS, Keen CL, Ahrén B. Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. Am J Physiol 1998; 274(5):R1482-1491.
15. Yarmohmmadi F, Rahimi N, Faghir-Ghanesefat H, Javadian N, Abdollahi A, Pasalar P, Jazayeri F, Ejtemaeemehr S, Dehpour AR. Protective effects of agmatine on doxorubicin-induced chronic cardiotoxicity in rat. Eur J Pharmacol 2017; 796:39-44.
16. Pieske B, Maier LS, Bers DM, Hasenfuss G. Ca2+ handling and sarcoplasmic reticulum Ca2+ content in isolated failing and nonfailing human myocardium. Circ Res 1999; 85(1):38-46.
17. Braveny P, Kruta V. Dissociation of 2 factors: restitution & potentiation in the effect of the pause on the amplitude of myocardial contraction. Arch Int Physiol Biochim 1958; 66(4):633-652.
18. Marwick TH. Diabetic heart disease. Postgrad Med J 2008; 84(990):188-192.
19. Suetta C, Hvid LG, Justesen L, Christensen U, Neergaard K, Simonsen L, Ortenblad N, Magnusson SP, Kjaer M, Aagaard P. Effects of aging on human skeletal muscle after immobilization and retraining. J Appl Physiol 2009; 107(4):1172-1180.
20. Gimenes R, Gimenes C, Rosa CM, Xavier NP, Campos DHS, Fernandes AAH, Cezar MDM, Guirado GN, Pagan LU, Chaer ID. Influence of apocynin on cardiac remodeling in rats with streptozotocin-induced diabetes mellitus. Cardiovasc Diabetol 2018; 17(1):15.
21. Dalla Vecchia LA, Bussotti M. Exercise training in pulmonary arterial hypertension. J Thorac Dis 2018; 10(1):508-521.
22. Janssen PM. Myocardial contraction-relaxation coupling. Am J Physiol Heart Circ Physiol 2010; 299(6):H1741-1749.
23. Chen C, Zou LX, Lin QY, Yan X, Bi HL, Xie X, Wang S, Wang QS, Zhang YL, Li HH. Resveratrol as a new inhibitor of immunoproteasome prevents PTEN degradation and attenuates cardiac hypertrophy after pressure overload. Redox Biol 2019; 20:390-401.
24. Zhao J, Ma HJ, Dong JH, Zhang LP, Liu HL, Wang QS. Electrophysiological effects of resveratrol on guinea pig papillary muscles. Sheng Li Xue Bao 2004; 56(6):708-712.
25. Bennett BT, Mohamed JS, Alway SE. Effects of resveratrol on the recovery of muscle mass following disuse in the plantaris muscle of aged rats. PLoS One 2013; 8(12):e83518.
26. Zhao X, Wang X, Wang J, Yuan J, Zhang J, Zhu X, Lei C, Yang Q, Wang B, Cao F. A Peptide-Functionalized Magnetic Nanoplatform-Loaded Melatonin for Targeted Amelioration of Fibrosis in Pressure Overload-Induced Cardiac Hypertrophy. Int J Nanomedicine 2020; 15:1321-1333.
27. Xiao H, Ma X, Feng W, Fu Y, Lu Z, Xu M, Shen Q, Zhu Y, Zhang Y. Metformin attenuates cardiac fibrosis by inhibiting the TGFbeta1-Smad3 signalling pathway. Cardiovasc Res 2010; 87(3):504-513.
28. Karimfar MH, Rostami S, Haghani K, Bakhtiyari S, Noori-Zadeh A. Melatonın Allevıates Bleomycın-Induced Pulmonary Fıbrosıs In Mıce. J Biol Regul Homeost Agents 2015; 29(2):327-334.
29. Massella D, Leone F, Peila R, Barresi AA, Ferri A. Functionalization of Cotton Fabrics with Polycaprolactone Nanoparticles for Transdermal Release of Melatonin. J Funct Biomater 2017; 9(1).
30. Abete P, Bianco S, Calabrese C, Napoli C, Cacciatore F, Ferrara N, Rengo F. Effects of melatonin in isolated rat papillary muscle. FEBS Lett 1997; 412(1):79-85.

31. Cook JS, Sauder CL, Ray CA. Melatonin differentially affects vascular blood flow in humans. Am J Physiol Heart Circ Physiol 2011; 300(2):H670-674.
32. Lamont KT, Somers S, Lacerda L, Opie LH, Lecour S. Is red wine a SAFE sip away from cardioprotection? Mechanisms involved in resveratrol- and melatonin-induced cardioprotection. J Pineal Res 2011; 50(4):374-380.
33. Floreani M, Napoli E, Quintieri L, Palatini P. Oral administration of trans-resveratrol to guinea pigs increases cardiac DT-diaphorase and catalase activities, and protects isolated atria from menadione toxicity. Life Sci 2003; 72(24):2741-2750.
34. Kurcer Z, Sahna E, Olmez E. Vascular reactivity to various vasoconstrictor agents and endothelium-dependent relaxations of rat thoracic aorta in the long-term period of pinealectomy. J Pharmacol Sci 2006; 101(4):329-334.
35. Ravens U, Link S, Gath J, Noble MI. Post-rest potentiation and its decay after inotropic interventions in isolated rat heart muscle. Pharmacol Toxicol 1995; 76(1):9-16.
36. Wohlfart B. Relationships between peak force, action potential duration and stimulus interval in rabbit myocardium. Acta Physiol Scand 1979; 106(4):395-409.
37. Cosyns B, Droogmans S, Weytjens C, Lahoutte T, Van Camp G, Schoors D, Franken PR. Effect of streptozotocin-induced diabetes on left ventricular function in adult rats: an in vivo Pinhole Gated SPECT study. Cardiovasc Diabetol 2007; 6:30.

TABLES AND FİGURES

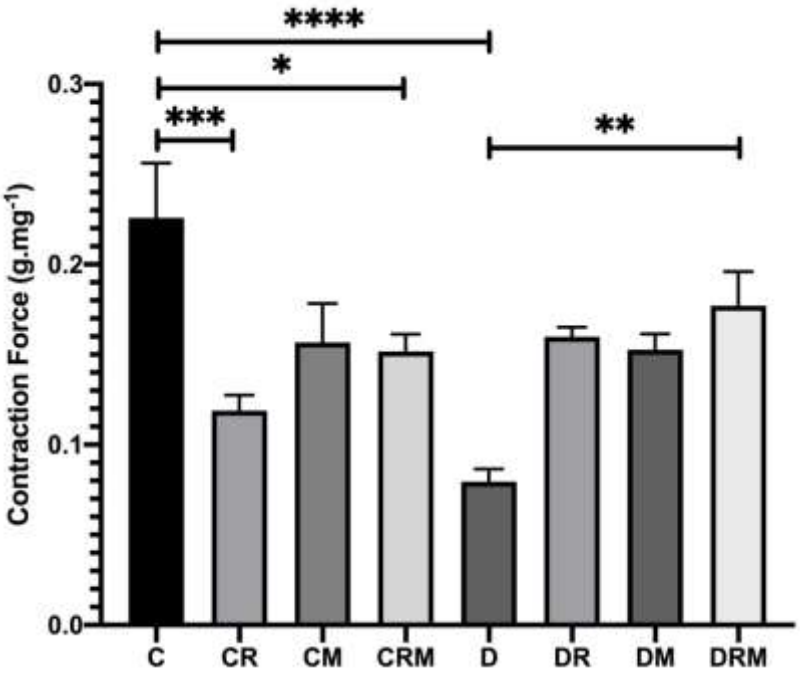
Table 1. Blood glucose levels (mmol/I) of all experimental groups

Groups (N=6)	Blood Glucose Levels (mmol/l)
C	5.10 ± 0.13^{b}
CR	4.97 ± 0.10^{b}
CM	4.88 ± 0.12^{b}
CR+M	4.94 ± 0.11^{b}
D	25.20 ± 1.57^{a}
DR	24.97 ± 0.69^{a}
DM	25.07 ± 0.60^{a}
DR+M	22.96 ± 0.36^{a}

Blood glucose values (mmol/I) values are given as mean ± SEM. a, b: The difference between the means of groups with different letters in the same column is significant (a > b) ($p < 0.05$). C, Control group; CR, Control Resveratrol group; CM, Control Melatonin group; CRM, Control Resveratrol Melatonin group; D, Diabetes group; DR, Diabetes Resveratrol group; DM, Diabetes Melatonin group; DRM, Diabetes Resveratrol Melatonin group.

Figure 1: (**A**) Contraction force (CF) (g.mg-1), (**B)** Contraction time (CT) (ms) recording values of all subjects created with 0.2 Hz stimuli. Findings regarding the responses to stimuli at a frequency of 0.2 Hz are given as mean ± standard error. ~~* indicates the degree of significance between the groups at the p<0.05 level.~~ *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. C, Control group; CR, Control Resveratrol group; CM, Control Melatonin group; CRM, Control Resveratrol Melatonin group; D, Diabetes group; DR, Diabetes Resveratrol group; DM, Diabetes Melatonin group; DRM, Diabetes Resveratrol Melatonin group.

A



B

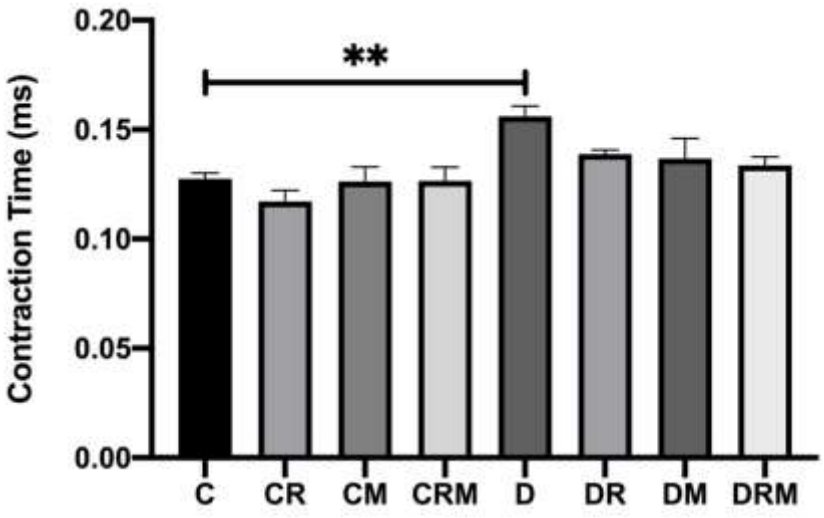


Figure 2. (**A, B, C**) Contraction Force (**D, E, F**) Contraction Time results of the stimulus frequency – contraction relationship. CF and CT responses to stimuli at different frequencies are given as mean ± standard error. (**A, B, C**) a, C and CR groups; b, C and CM groups; c, C and CRM groups; ~~groups~~ d, C and D groups; ~~groups~~ e, D and DR groups; f, D and DM groups; g indicates the significance between D and DRM groups ($p<0.05$), (**D, E, F**) d shows the significance between C and D groups ($p<0.05$). C, Control group; CR, Control Resveratrol group; CM, Control Melatonin group; CRM, Control Resveratrol Melatonin group; D, Diabetes group; DR, Diabetes Resveratrol group; DM, Diabetes Melatonin group; DRM, Diabetes Resveratrol Melatonin group.

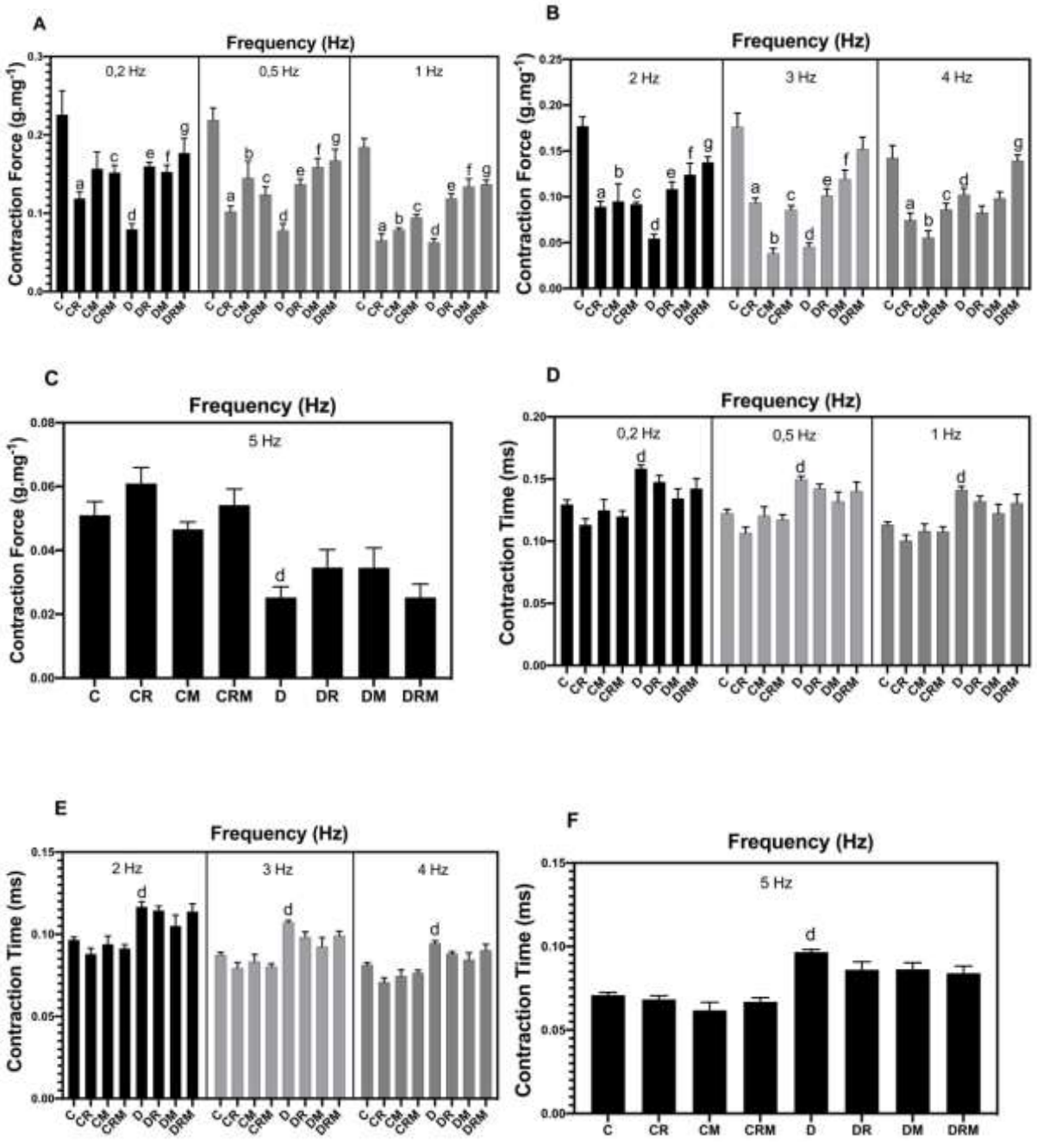
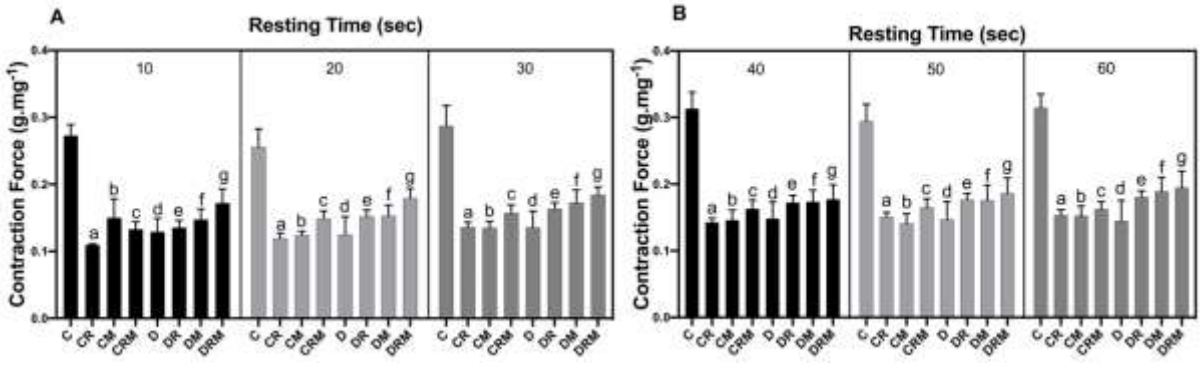
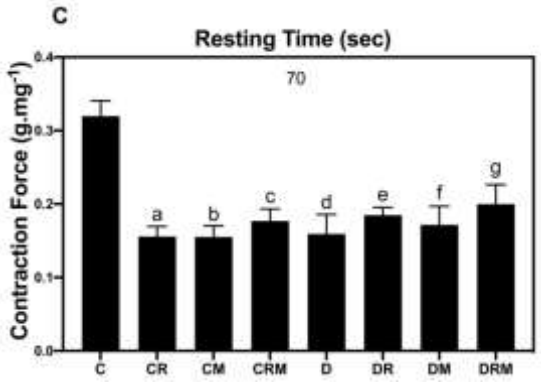


Figure 3A, B, C: CF values of the anticipatory stimulus-contraction relationship. CF parameter responses to the predicted stimuli are depicted as mean ± standard error. a, C and CR groups; b, C and CM groups; c, C and CRM groups; ~~groups~~ d, C and D groups; ~~in groups~~ e, D and DR groups; f, D and DM groups; g indicates the significance between the D and DRM groups ($p<0.05$). C, Control group; CR, Control Resveratrol group; CM, Control Melatonin group; CRM, Control Resveratrol Melatonin group; D, Diabetes group; DR, Diabetes Resveratrol group; DM, Diabetes Melatonin group; DRM, Diabetes Resveratrol Melatonin group.

501



502



503

504

Figure 4A, B, C: CT values of the pre-pending stimulus-contraction relationship. Response values of the CT parameter to the pre-pending stimuli are shown as mean ± standard error. d shows the significance between groups C and D ($p<0.05$). C, Control group; CR, Control Resveratrol group; CM, Control Melatonin group; CRM, Control Resveratrol Melatonin group; D, Diabetes group; DR, Diabetes Resveratrol group; DM, Diabetes Melatonin group; DRM, Diabetes Resveratrol Melatonin group.

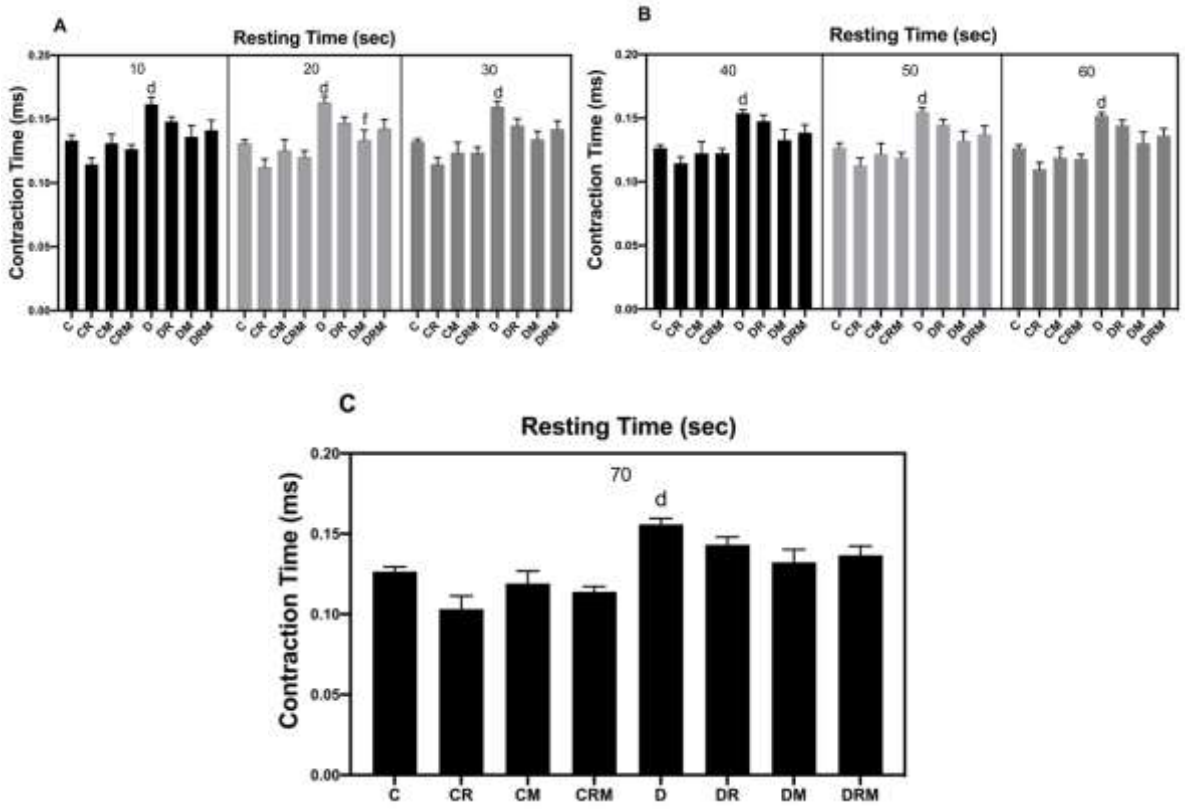


Figure 5. Hematoxylin Eosin staining scale: 20µm, 40x magnification (black arrow: intracytoplasmic vacuole). C, Control group; CR, Control Resveratrol group; CM, Control

Melatonin group; CRM, Control Resveratrol Melatonin group; D, Diabetes group; DR, Diabetes Resveratrol group; DM, Diabetes Melatonin group; DRM, Diabetes Resveratrol Melatonin group.