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VERSITA

ACTA FACULTATIS PHARMACEUTICAE UNIVERSITATIS COMENIANAE

CARVEDILOL AND PYCNOGENOL® IMPROVE THE FUNCTION OF DIABETIC HEARTS IN RATS

KARVEDILOL A PYCNOGENOL® ZLEPŠUJÚ FUNKCIU DIABETICKÝCH SŔDC U POTKANOV

Original research article

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Abstract We observed the changes in electrical activity, biometric and haemodynamic parameters of hearts in animals with experimental diabetes mellitus (DM). As well the effect of carvedilol, Pycnogenol® and its combination with carvedilol on DM heart function was tested. DM was induced by streptozotocin over three sequential days at a dose of 25 mg/kg body weight i.p. We started therapy by suspension of carvedilol, Pycnogenol® and their combination for six weeks. Blood pressure was measured using tail cuff plethysmography. ECG, haemodynamic and biometric parameters were measured in isolated hearts perfused according to the Langendorff. DM rats had increased systolic arterial blood pressure, thicker free wall of left ventricle but weakened myocardial contractility compared with controls. In contrast to controls, electrophysiological parameters showed prolonged QT interval and increased incidence of dysrhythmias in DM rats. The Pycnogenol® administration induced regression of left ventricular hypertrophy, improved left ventriculi contraction and increased coronary flow; however, it did not improve the electrical activity of the myocardium compared with DM ones. Carvedilol also reversed the myocardial remodelling, shortened the duration of QT interval and suppressed the incidence of dysrhythmias. The common combination of drugs improved biometric and haemodynamic parameters compared with DM animals, however, not so significantly as monotherapy. On the other hand, the combination of carvedilol and Pycnogenol® significantly reduced the duration of the QT interval and shortened the incidence of dysrhythmias. We can conclude that the administration of Pycnogenol® effectively improved haemodynamic parameters, and carvedilol affected biometric parameters and also electrical parameters in DM animals. We observed the marked synergic effect of the combination of both drugs on the electrical activity of myocardium. This combination shortened the most pathologically prolonged QT interval and reduced the number of dysrhythmias.

Slovak abstract Sledovali sme zmeny elektrickej aktivity, biometrických a hemodynamických parametrov myokardu u diabetických zvierat a následne testovali účinok karvedilolu, Pycnogenolu® a ich kombinácie na funkciu diabetických sŕdc. Diabetes mellitus (DM) bol navodený tri dni po sebe dávkou streptozotocínu 25 mg/kg, i.p. Látky sa podávali šesť týždňov p.o. Tlak krvi bol meraný metódou „tail cuff". Elektrokardiogram a hemodynamické parametre sa zaznamenávali na izolovaných srdciach, perfundovaných metódou podľa Langendorffa. Na izolovaných srdciach sme merali aj biometrické parametre. Diabetické zvieratá mali zvýšený systolický arteriálny tlak. DM vyvolal signifikantné zhrubnutie ľavej komory a oslabenie kontraktility myokardu. Elektrofyziologické parametre ukázali predĺžený QT interval a zvýšenú incidenciu porúch rytmu. Aplikácia Pycnogenolu® viedla k regresii hypertrofie ľavej komory, zlepšeniu kontrakcie a zvýšeniu koronárneho prietoku, nezlepšila však elektrickú aktivitu myokardu. Karvedilol tiež úspešne zvrátil remodeláciu myokardu, skrátil trvanie QT intervalu a znížil incidenciu dysrytmií. Spoločná kombinácia týchto látok zlepšilahodnoty biometrických a hemodynamických parametrov oproti diabetickým zvieratám, no nie tak významne ako ich samostatné podanie. Naopak vzájomná kombinácia karvedilolu a Pycnogenolu® výrazne skrátila trvanie QT intervalu a znížila počet dysrytmií. Na záver môžeme zhrnúť, že samotné podávanie Pycnogenolu® najefektívnejšie zlepšilo hemodynamické parametre, karvedilol najlepšie ovplynil biometrické parametre a čiastočne aj elektrickú aktivitu diabetických sŕdc.Výrazné synergické účinky priniesla kombinácia oboch liečiv len pri ovplyvnení elektrickej aktivity myokardu, kde najvýznamnejšie skrátila patologicky predĺžený QT interval a znížila tak počet dysrytmií.

Keywords *diabetes mellitus – ECG – carvedilol – Pycnogenol® – rat*

Kľúčové slová: *diabetes mellitus – EKG – karvedilol – Pycnogenol® – potkan*

INTRODUCTION

Cardiovascular diseases are the primary cause of morbidity and mortality among patients with diabetes mellitus (DM). Diabetics thus have a long term cardiovascular risk similar to that observed among non-diabetic patients with previous myocardial infarction (Emre et al., 2010). Diabetic cardiomyopathy has been defined as the presence of myocardial abnormalities in the absence of coronary artery disease, hypertension or other significant etiology (Bauters et al., 2003). The mechanism of diabetic cardiomyopathy is not completely understood (Emre et al., 2010). It has been

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postulated that endothelial dysfunction, endomyocardial fibrosis, direct toxic effect of hyperglycaemia on cardiomyocytes and autonomic neuropathy play an important role. These cardiovascular complications compromise cardiac performance ultimately resulting in cardiac failure (Yu et al., 2007). Despite intensive medical research on heart failure caused by DM and new medical treatments of these patients, left ventricle (LV) dysfunction still remains a major cause of mortality in diabetic patients and warrants further testing of novel therapeutic approaches (Grimm et al., 2002).

Carvedilol is a nonselective β-adrenoceptor-blocking agent and a selective α_1-adrenoceptor blocker. So far, it has been widely used in the treatment of heart failure, hypertension with or without DM. It has been suggested that carvedilol may provide greater benefit than traditional β-blockers in chronic heart failure because of its antioxidant actions that synergise with its nonspecific α_1-and β-blocking effects. Although, it has been reported that carvedilol had no negative effects including insulin sensitivity besides glucose and lipid metabolisms (Huang et al., 2007).

Herbal extracts, vitamins, antioxidants and minerals are also known as nutraceuticals or nutritional supplements. They are important supplements to the classical treatment of DM and insulin resistance with hypoglycaemic and antioxidant properties, for example (Kinsel & Straus, 2003). Pycnogenol® is a standardised water-soluble extract of the bark of French maritime pine (Pinus pinaster ssp. atlantica) with significant antioxidant and hypoglycaemic properties. Pycnogenol® is used as a nutritional supplement and interacts with free radicals, alleviating damage to the cell by scavenging radicals and by binding them to proteins (Packer et al., 1999). Pycnogenol® showed positive effects on neuronal activity in diabetic neuropathy (Jankyova et al., 2009) and on cardiac performance in diabetic cardiomyopathy (Klimas et al., 2010) too. Similarly in humans, Pycnogenol® diminished diabetic microangiopathy and reduced cardiovascular risk factors (Packer et al., 1999).

We were interested if the combination of the drug carvedilol and nutritional supplement Pycnogenol® is more effective than their individual administration on myocardial function of isolated hearts in rats with experimental diabetes.

ANIMALS AND METHODS

Animals

Three-month-old male Wistar rats obtained from the breeding station Dobra Voda (Slovak Republic – SR) were used for experiments. All animals were allowed to adapt to the housing conditions with free access to food and tap water for at least seven days. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published in the Collection of Laws of the Slovak Republic (Z.z. SR) No. 289/2003 and was approved by the Ethics Committee of the Faculty of Pharmacy CU and by the State Veterinary and Food Administration, SR (protocol No: Ro-2222/06-221).

Induction of diabetes and treatment

After randomisation of experimental animals, DM was induced by the administration of streptozotocine i.p. (STZ, Sigma, St. Louis, USA) three days consecutively (25 mg/kg b.w.). STZ was dissolved in citrate buffer (0.1 mol/l, pH = 4.5). The animals were fasting twelve hours before the administration of STZ. After the diabetes induction, animals were kept for seven days without any treatment for the development of DM. Arterio-venous blood from the rat tail vein of the rats was taken for the assessment of blood glucose levels. Non-diabetic animals were excluded from the experiment.

Control animals without diabetes (Control, n = 7) and group of diabetic animals without treatment (Diabetic, n = 7) received p.o. vehicle. The other three groups of diabetic animals were administered Pycnogenol® (n = 6, 20 mg/kg, Generica s.r.o., Piestany), carvedilol (n = 5, 0.09 mg/kg, Carvedilol – TEVA 6.25 mg/kg) and a combination of carvedilol and Pycnogenol® (n = 5). These substances were given by the gastric gavage in water suspension form for 6 weeks.

Blood pressure measurement

The mean arterial blood pressure was measured by the tail-cuff method in conscious animals pre-warmed to 39°C. Blood pressure was measured by a non-invasive blood pressure module (NIBP Controller, ADInstruments, Spechbach, Germany) connected via a manometer and a Powerlab 8/30 data acquisition module (AD Instruments, Spechbach, Germany) to a computer (PS Tronic, Bratislava, Slovak Republic). For every data point, five recordings were analysed and mean values were calculated. Data analyses were performed using Chart 5 software for Windows (AD Instruments, Spechbach, Germany).

Haemodynamic, biometric and ECG parameters of isolated heart

Haemodynamic parameters were measured on the isolated hearts perfused according to the Langendorff. The abdominal cavity was opened and the rat was heparinised (500 IU, Heparin inj. 5000 I.U., Léčiva, Prague, Czech Republic) into vena cava inferior. After chest opening, the heart was separated and rapidly immersed into the cold (4°C) Krebs-Henseleit (K-H) solution where cannula was inserted into the aorta and fixed with a silk ligature. The heart was placed in an organ chamber in Langendorff apparatus and retrogradely perfused with K-H solution at constant pressure mode (90–100 cm H_20). K-H solution contained (in mM) NaCl – 118.0; KCl – 4.70; $MgSO_4 \times 7\ H_2O$ – 1.66; KH_2PO_4 – 1.18; $CaCl_2 \times H_2O$ – 2.00; $NaHCO_3$ – 15.00; glucose – 11.00 (all chemicals were from Centralchem, Bratislava, Slovak Republic) and saturated with 95% oxygen and 5% carbon dioxide (pH 7.35–7.4, temperature 36.5–37°C). Ventricular pressure was measured by means of a water-filled balloon inserted into the LV cavity through a left atrial incision. A balloon volume was adjusted to yield 9–10 mmHg of LV end-diastolic pressure. LV pressure was continuously monitored by electromanometer (LDP186 Tesla, Valašské Meziříčí, Czech

Republic) and recorded by Powerlab 8/30 (AD Instruments, Germany). LV developed pressure as a measure of cardiac contractility was calculated as a difference between the LV systolic and diastolic pressures. From isolated spontaneously beating hearts, one lead ventricular electrocardiogram was recorded using a pair of wire electrodes impaled into the LV free wall. At the end of experiment, the heart was placed into the cold K-H solution to stop beating. The mass of LV was estimated by measuring blotted wet LV weight. The thickness of left ventricular free wall was measured by sliding rule.

Data are expressed as mean × ± SEM. Statistical significance between groups was tested using Student´s t-test and a probability value $p \leq 0.05$ was considered to be statistically significant.

RESULTS

Preprandial blood glucose level was about 7 mmol/l and postprandial glucose was 16 mmol/l in the DM group. In the control group, the value of preprandial glucose was 6 mmol/l, and postprandial blood glucose 9 mmol/l.

Systolic blood pressure was increased in DM animals about 16% compared with control. The hearts isolated from DM rats were characterised by increased left ventricular mass and LV wall thickening compared with controls (Table 1, Figure 1). The hearts in DM group showed impaired LV contraction (LVPs-d) and reduced coronary flow through the vessels for about 19% (Table 1, Figure 2). Duration QT interval was overextended for about 11% and QTc interval for about 10% in DM hearts compared with controls (Table 2). The value of the QT interval was corrected for the frequency according to the modified Bazett formula (Izraelová et al, 2006). It is generally accepted that prolonged QT intervals reveal depolarisation abnormalities that increase the risk of ventricular dysrhythmias. In hearts isolated from DM rats, 336 events during 75-minute lasting perfusion were identified (ventricular premature beats, bigeminy, trigeminy, salvos; Figure 3).

Pycnogenol® decreased the values of biometric parameters, significantly increased contraction for about 96%, and improved coronary flow for about 54% compared with DM group (Table 1, Figure 1). In contrast to DM rats, Pycnogenol® did not change systolic blood pressure (Figure 2). Pycnogenol® also did not change significantly the duration of QT interval compared with DM hearts; it, however, induced the majority of episodes of dysrhythmias (348 dysrhythmias) from all groups compared with DM group (Table 2, Figure 3).

The administration of β-blocker carvedilol significantly reduced thickness of LV and increased myocardial contraction compared with DM group (Table 1, Figure 1).Carvedilol significantly decreased systolic blood pressure (Figure 2). The application of carvedilol significantly shortened the duration of QT interval for about 10% and decreased amount of dysrhythmias (127 dysrhythmias) compared with DM hearts (Table 2, Figure 3).

The combination of Pycnogenol® and carvedilol decreased significantly the thickness of LV and blood pressure and improved the haemodynamic function of heart compared with DM group, but not so effectively as in monotherapy (Table 1, Figures 1, 2). The combination of these drugs reduced QT interval the most effectively for about 12% in comparison with DM hearts. We observed less amount of dysrhythmias after the administration of combination of carvedilol and Pycnogenol® (122 dysrhythmias) compared with DM group (Table 2, Figure 3).

DISCUSSION

DM is associated with many complications in organisms due to hyperglycaemia. The reduction of hyperglycaemia is a key factor of DM pharmacotherapy. There is growing interest in herbal remedies due to the side effects associated with the therapeutic agents for the treatment of DM. In the therapy of diabetes, except oral antidiabetics, drugs from other groups are used (ACE inhibitors, β-blockers), especially to prevent damage to the cardiovascular system. Carvedilol exerts additional potent antioxidant and antiproliferative properties and has been shown to improve the prognosis of patients with heart failure, a situation with continuous remodelling and ongoing apoptosis (Ruffolo et al., 1993, Packer et al., 1996).

One of the types of an experimental model of diabetes is the model based on the application of STZ (Thulesen et al., 1997). In our experiment, the administration of STZ rats caused an increase in preprandial and postprandial glycaemic control and weight loss of animals. The other authors (Grimm et al., 2002, Huang et al., 2006) using this experimental model of diabetes showed the same data. DM is a major risk for the development

Table 1. Biometric and hemodynamic parameters of left ventricle.

Left ventricle	Control	D	DP	DC	DPC
	n=7	n=7	n=6	n=5	n=5
weight (g)	0.88 ± 0.02	0.94 ± 0.02 *	0.76 ± 0.01 ###	0.77 ± 0.01 §§§	0.82 ± 0.03 $
wall thickness (mm)	3.37 ± 0.06	3.61 ± 0.08 *	3.52 ± 0.07	3.33 ± 0.07 §§	3.38 ± 0.04 $
LVPs-d (mmHg)	20.57 ± 4.43	14.61 ± 2.41*	28.72 ± 4.39 #	26.19 ± 3,07 §	21.62 ± 3.30 $
CF (ml/min)	6.93 ± 0.48	5.60 ± 1.31	8.60 ± 0.99 ##	7.22 ± 1.21 §	6,81 ± 1.41

Legend: D = diabetes, DP = diabetes+Pycnogenol®, DC = diabetes + carvedilol, DPC = diabetes + Pycnogenol® + carvedilol.
*Data are expressed as x ± SEM, $p < 0.05$ for *D vs control, #DP vs D, §DC vs D, $DPC vs D, $p < 0.01$ for ##DP vs D, §§DC vs D , $p < 0.001$ for ###DP vs D, §§§DC vs D. LVPs-d = left ventricular pressure, s – systola, d – diastola, CF – coronary flow.*

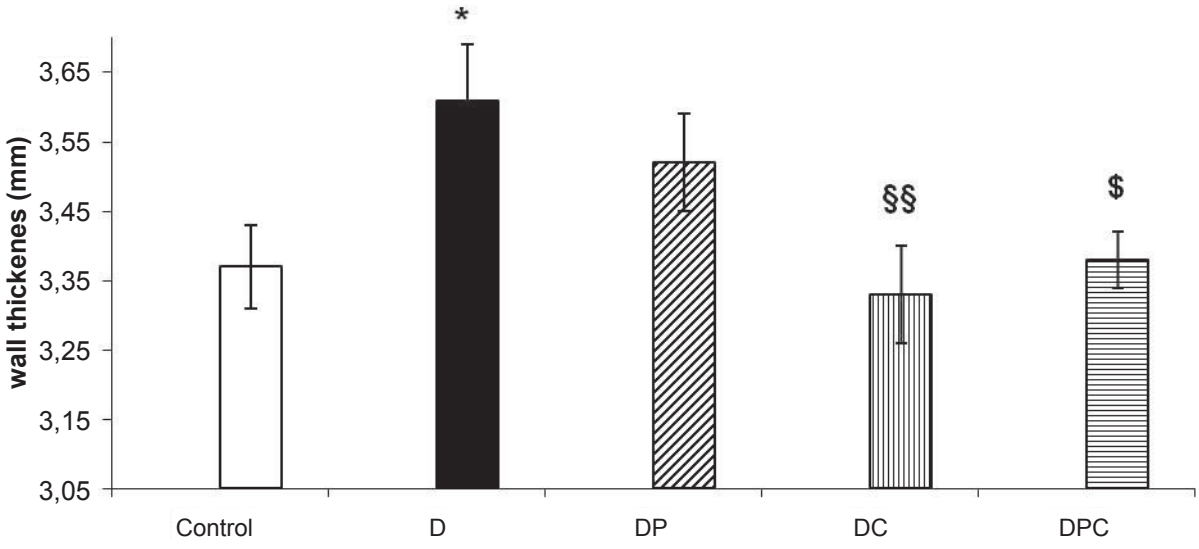

Figure 1. The thickenss of left ventricle. Legend is the same as in Table 1. Data are expressed as x ± SEM, p< 0.05 for *D vs control, $ DPC vs D, p< 0.01 for §§ DC vs D.

of cardiovascular disorders, especially for hypertension. In our experiment, we observed a significant increase in systolic arterial pressure in DM animals. This increase in blood pressure in DM animals may result from accumulation of collagen in the wall of blood vessels or the effect of atherosclerosis. Jankyova et al. (2013) confirmed the changes in the collagen amount as increased ratio of collagen I/III in vessels of untreated DM animals, which led to elevation of vascular rigidity. The increase blood pressure can also relate to sustained hyperglycaemia, which activates the renin–angiotensin system (Miller et al., 1996). Hosseini et al. (2001) administered Pycnogenol® to hypertensive patients and found out that Pycnogenol® decreased systolic pressure. In our study, we observed that Pycnogenol® did not change systolic blood pressure in comparison with DM group. In contrast to DM group, carvedilol significantly decreased systolic blood pressure in monotherapy and in combination with Pycnogenol®. The mechanism of this carvedilol´s effect is multifactorial and is involved in decreased cardiac output, decreased renin activity and altered sensitivity of β-adrenergic receptors (Kuželová , 2008).

DM is independently associated with abnormal left ventricular relaxation, which is similar to impaired relaxation in hypertension. LV remodelling includes enlargement of left ventricular mass, increased left ventricular wall thickness and deterioration of myocardial function (Liu et al., 2001). In our experiment, we observed an increase in the left ventricular mass and left ventricular thickness in DM group. Similar results were reflected in the experiments conducted by Goyal et al. (2011). The administration of Pycnogenol® positively influenced pathological myocardial remodelling because the weight and thickness of LV were reduced. Maimoona et al. (2010) in their work attributed this fact to the effect of a reduction in collagen, fibrosis and angiotensin II in the heart. Carvedilol was able to successfully reverse LV remodelling in monotherapy and in combination with Pycnogenol®. Grimm et al. (2002) supposed that carvedilol induces regression of LV hypertrophy and attenuates the overexpression of extracellular matrix proteins in diabetic cardiomyopathy, probably due to its additive antiproliferative and antioxidant properties.

Preclinical stage of DM cardiomyopathy is most often demonstrated by left ventricular dysfunction which can lead to heart failure (Battiprolu et al., 2010). The previous study of Spindler et al. (1999) has shown that diabetic cardiomyopathy is characterised by decreased contractility and impaired relaxation. In our experiment, DM hearts had weakened contraction and reduced coronary flow compared with control hearts. This decrease can be related to the development of coronary macroangiopathy. In contrast to DM hearts, the administration of Pycnogenol® significantly improved contraction. This effect may be related to antioxidant properties and the ability to induce endothelial NO production (Drobná et al., 2011). The reduction of fibrosis in the heart can also contribute to

Table 2. QT and QTc parameters of isolated hearts.

Isolated heart	Control	D	DP	DC	DPC
	n=7	n=7	n=6	n=5	n=5
QT (ms)	90.19 ± 2.95	100.17 ± 10.07*	101.13 ± 5.92	90.06 ± 1.32 §	88.25 ± 2.49 §
QTc (ms)	70.46 ± 2.83	74.61 ± 11.38*	77.20 ± 4.38	62.26 ± 3.53 §	57.40 ± 4.30 §

*Legend is the same as in Table 1. Data are expressed as x ± SEM, p< 0.05 for * D vs control, p< 0.05 for § DPC vs D, § DC vs D.*

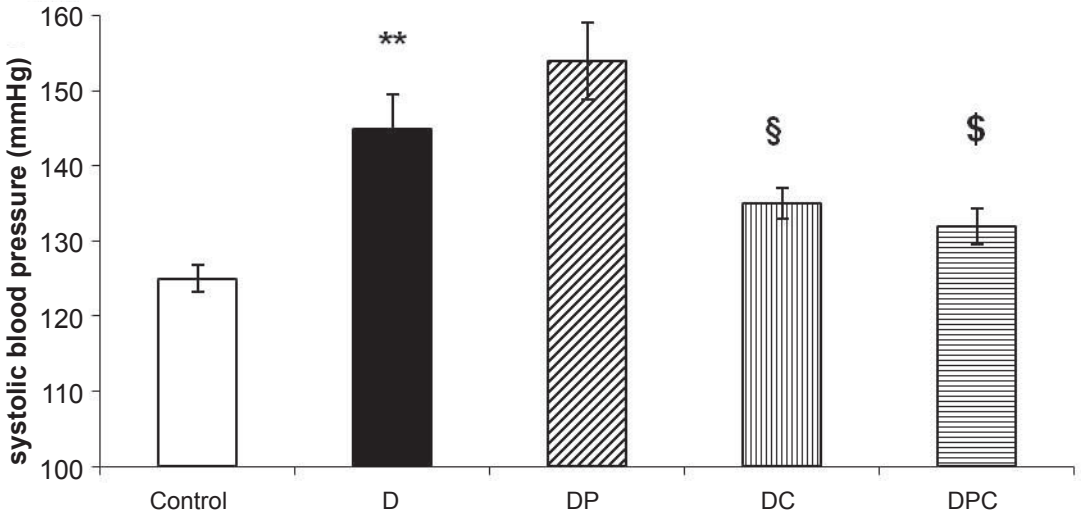

Figure 2. The systolic blood pressure. Legend is the same as in Table 1. Data are expressed as x ± SEM, p< 0.05 for *D vs control, $ DPC vs D, p< 0.01 for §§ DC vs D.

improved haemodynamic function. Carvedilol also improved contraction and increased coronary flow compared with DM hearts, but not as effectively as Pycnogenol®. This effect is probably due to its antioxidant and vasodilatory properties mediated by adrenergic blockade (Antelava et al., 2009). The combination of carvedilol and Pycnogenol® improved contraction and coronary flow compared with DM heart, but not so significantly like monotherapy.

DM is also associated with changes in the electrical activity of the heart. Abnormal QT prolongation is usually associated with dysrhythmias. We recorded a prolonged QT interval in DM hearts. It can be explained by the fact that DM animals suffered from polyuria. Excessive urination causes potassium loss (Kitabchi & Wall, 1995), which may be responsible for an extended period of depolarisation and thereby QT prolongation. We also found that DM hearts showed an increased incidence of rhythm disturbances. Pycnogenol® did not change the duration of QT interval compared with DM hearts. These results are in concordance with results in the study by Jankyova et al. (2012). Pycnogenol® only slightly increased the total number of dysrhythmias compared with the DM group. However, we observed an increase in serious rhythm disorders such as trigeminies and salvos. In contrast to DM hearts, carvedilol significantly shortened the duration of the QT interval. A possible explanation is that carvedilol blocks the potassium current IKr, which participates in depolarisation prolonging of the heart cells or effect of the blockade of Ca^{2+} channel L-type (El-Sherif & Turitto, 2005). The hearts premedicated by Pycnogenol® and carvedilol had the most shortened QT interval compared with DM group of animals. In this group, we also recorded the minimum of dysrhythmias. We predict that on this effect participated more carvedilol.

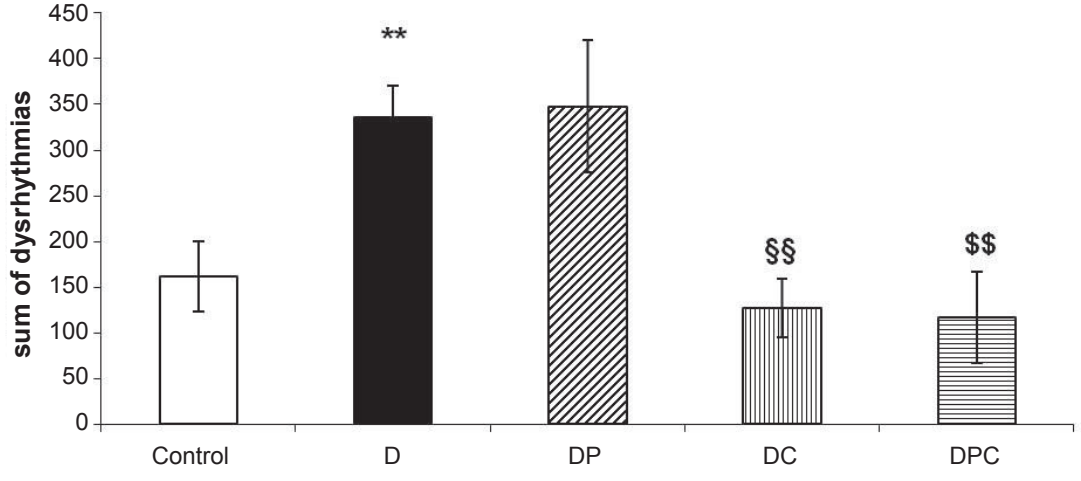

Figure 3. The incidence of dysrhythmias. Legend is the same as in Table 1. Data are expressed as x ± SEM, p< 0.01 for **D vs control, $$ DPC vs D, §§ DC vs D.

CONCLUSION

The administration of STZ resulted in DM in animals. Experimental DM was characterised by increased preprandial and postprandial blood glucose in animals. The DM hearts were characterised by LV hypertrophy and LV dysfunction, prolongation of QT interval and increased incidence of dysrhythmias. The administration of Pycnogenol® led to improving of values of haemodynamic parameters – contraction and coronary flow. The values of electrophysiological parameters and incidence of rhythm disturbances of isolated hearts were not improved. Carvedilol successfully reversed the myocardial remodelling – thickness of the left ventricular wall. Isolated hearts showed shortened QT interval and lower incidence of dysrhythmias compared with the DM group. In contrast to DM hearts, the combination of Pycnogenol® and carvedilol improved the biometric and haemodynamic parameters. However, this improvement was not as effective as monotherapy with the individual substances. On the other hand, the combination of both drugs significantly ameliorated electrical functions of DM hearts.

We can conclude that the β-blocker carvedilol and Pycnogenol® improved the myocardial function in DM animals. The effects of these substances on the values of biometric and haemodynamic parameters were more effective in monotherapy than in combination. The combination improved only the electrical parameters.

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