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Morphological and morphometric studies of the aorta, pulmonary trunk, and heart of streptozotocin-induced diabetic Wistar rats

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Micro-anatomical changes in the aorta, pulmonary trunk, and left ventricle of Wistar rats were studied after the administration of streptozotocin. Twenty adult Rattus norvegicus *were randomly assigned into two groups (control and diabetic) of ten rats each. Diabetes mellitus was experimentally induced in the diabetic group of rats by daily intra-peritoneal administration of multiple doses of 40 mg/kg streptozotocin dissolved in 0.1 M sodium citrate buffer for five consecutive days. The control group was given the equivalent volume of citrate buffer. The animals were monitored for four weeks after streptozotocin administration. Post sacrifice, the left ventricle, aorta, and pulmonary trunk were excised, weighed, and fixed by immersion in 10% formol saline. The tissues were processed for paraffin embedding, and sections of 6 µm thickness were produced and stained with H & E for general histological observations, and Verhoeff-van Gieson elastic fibre stain to demonstrate elastic fibres in these cardiovascular structures. The data obtained were analyzed with descriptive and inferential statistics. Histopathological and morphometric examinations of the stained sections showed a significant increase in the thickness of the tunica intima of aorta ($t = -7.49$; $df = 9$; $p < 0.05$) and pulmonary trunk ($t = -10.81$; $df = 9$; $p < 0.05$) in diabetic rats (14.59 ± 1.189 µm and 11.307 ± 0.863 µm, respectively) when compared to that of the control group (3.62 ± 0.353 µm and 3.22 ± 0.244 µm, respectively). In addition, the distribution of elastic and collagen fibres was sparse in the hearts of the diabetic group when compared to that of the control group. The findings of this study demonstrated that diabetes mellitus might cause some alterations in the microanatomy of cardiovascular structures.* (Folia Morphol 2009; 68, 4: 207–214)

Key words: diabetes mellitus, aorta, pulmonary trunk, left ventricle

INTRODUCTION

Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signalling, pathways, and the defect usually results from pancreatic beta-cell deficiency and/or a deficiency of insulin [13, 16]. This disease causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease, and heart disease. Cardiovascular disease is one of the major causes of death in diabetic patients [1, 38]. Since heart is one of the most important target tissues of diabetes, the increased rate of mortality and morbidity of these

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patients has been attributed to diabetic cardiomyopathy [10, 12]. Furthermore, growing evidence showing impaired diastolic performance followed by depressed systolic function in diabetic patients supports this hypothesis. Although it has been recognized for many years that diabetes mellitus is associated with cardiac dysfunction caused by cardiomyopathy without significant coronary artery disease, the pathogenesis of diabetic cardiomyopathy remains unclear.

Increased production of reactive oxygen species (ROS), as well as nitrogen species, is a well-known phenomenon in hyperglycaemic conditions. Although the aetiology of the hyperglycaemia-induced cellular damage has not yet been clarified, free radicals and oxidative stress are thought to be the contributory factors underlying these abnormalities. The auto oxidation of glucose, the formation of glycation end products and the activation of NADPH oxidase have been suggested as possible sources of the augmented oxidative stress in diabetes. Thus, elevated free radical levels activate various subcellular signal transduction pathways, including abnormal gene expression, which may cause myocardial cell death. Related with these suggestions, increased production of thiobarbituric acid-reactive substances (TBARS), which is an index of oxidative stress and lipid peroxidation, has been reported in diabetic hearts [2, 17, 18, 34].

Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, possesses a pancreatic beta-cell cytotoxic effect [36]. Streptozotocin has been widely used to induce diabetes mellitus in a variety of animals. This well-established model is characterized by insulin deficiency associated with insulin resistance [3]. It was reported that a single intravenous injection of STZ could cause increased plasma glucose levels, decrease in body weight, and 17% mortality in rats [3]. STZ causes degeneration and necrosis of pancreatic beta cells [27, 35]. Although the mechanism of the beta cell cytotoxic action of STZ is not fully understood, experimental evidence has demonstrated that some of its deleterious effects are attributable to induction of metabolic processes which lead to an increase in the generation of ROS [6]. Apart from production of ROS, STZ also inhibits free radical scavenger-enzymes [20]. The superoxide radical has been implicated in lipid peroxidation, DNA damage, and sulfhydryl oxidation [26, 33].

Cardiovascular disorder is implicated as a major cause of death in diabetic patients, thus this study aimed at investigating the microarchitectural aspect of the cardiovascular disorder following induction of diabetes under laboratory conditions.

MATERIAL AND METHODS

Care and management of animals

Twenty healthy adult male Wistar rats (*Rattus norvegicus*), weighing between 150 g and 250 g, were used for the experiment. They were kept in individual cages under natural light and dark cycles at room temperature. They were maintained on standard rat pellets (Ladokun feeds, Ibadan, Nigeria) and water given *ad libitum.* The animals were randomly assigned into two groups (control and diabetic) of ten rats each. There was a pre-experimental period of four weeks, during which the body weight, blood glucose level, and serum lipid profiles were monitored in the animals. The rats received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health [28].

Experimental induction of diabetes

Diabetes mellitus was induced in the diabetic group of rats by intraperitoneal administration of multiple low doses of streptozotocin (Sigma, St. Louis, USA) (40 mg/kg body weight) dissolved in freshly prepared 0.1 M sodium citrate buffer (pH 6.3) for five days, while the animals in the control group were given the equivalent volume of citrate buffer intraperitoneally. The rats were fasted overnight before STZ administration. The daily body weight and the weekly blood glucose level were monitored in the animals for the next four weeks.

Determination of body weight and blood glucose level

The body weight of the animals was measured using a top loader weighing balance. Blood samples were obtained from the tail vein of the animals, and their fasting blood glucose level was determined in mmol/L using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany). The animals were fasted for a period of 16 hours before their blood glucose level was measured.

Surgical procedures

A mid-line incision was made in the thoracic wall of the rats under slight anaesthesia using pentobarbital (6.4 mg/100 g body weight i.m.), and the

heart, aorta, and pulmonary trunk were removed and weighed on a top loader sensitive balance (Mettler Toledo, Germany). The aorta was isolated from the systemic circulatory tract by cutting it through its commencement at the ascending aorta (as it comes out of the left ventricle) and its terminal end as it bifurcates into two common iliac arteries at the level of the L1 Vertebra, while the pulmonary trunk was isolated from the pulmonary circulatory tract by cutting through its commencement as it comes out of the right ventricle (RV) and its terminus as it bifurcates into the right and left pulmonary arteries. The left ventricle (LV) was isolated from the remaining part of the heart through incisions made in the coronary sulcus and interventricular groove and was weighed. The relative heart weight and the relative left ventricular weight was calculated as follows.

$$\text{Relative heart weight} = \frac{\text{Weight of the heart}}{\text{Body weight at sacrifice}} \times 100$$

$$\text{Relative left ventricular heart weight} = \frac{\text{Weight of the left ventricle}}{\text{Weight of the heart}} \times 100$$

Histological procedure

The excised tissues (LV, ascending aorta, and pulmonary trunk) were fixed in 10% formol saline by total immersion for 48 hours and were processed via the paraffin wax embedding method of Drury and Wallington [9]. Paraffin-embedded sections were cut at 6 μm and stained with (a) haematoxylin and eosin (HE) for histoarchitectural examination of the tissues and (b) Verhoeff-Van Gieson stain for demonstration of elastic and collagen fibres. The sections were examined under a Carl Zeiss research microscope (Axioskope 40, Germany) with a digital camera attached. Digital photomicrographs of the pancreatic sections were taken at various magnifications.

Histomorphometric analysis

All morphometric studies were carried out on an Olympus research microscope (Olympus WF10X, Japan) with a linear scale-ocular micrometer inserted into the eyepiece. The ocular micrometer was calibrated with a 1 mm stage micrometer (Graticles Tonbridge, Kent, England). Forty-eight histological stained sections were used for morphometric analysis; eight sections each of the aorta, pulmonary trunk, and left ventricle of the control and diabetic groups of the rats were examined at different magnifications to estimate the (i) diameter of the lumen, (ii) thickness of the entire wall, and (ii) thickness of the each of the tunica intima, media, and adventitia. With an ocular micrometer and a graticule of a calibrated linear scale, the major axis (a) and minor axis (b) at right angles to the major axis of the lumen of each of the aorta, pulmonary trunk, and left ventricle were measured at 40× magnification. The lumen diameter of the sections (D_i) was calculated from the equation $D_i = \sqrt{ab}$ [37]. The wall to lumen ratio of the vessels was calculaed as $\frac{\text{Thickness of the wall}}{\text{Diameter of the lumen}}$.

Using a calibrated ocular micrometer, the thickness of the entire wall of each of the aorta, pulmonary trunk, and left ventricle was measured in μm at 400× magnification while the thickness of each of the tunica intima, media, and adventitia of the aorta and pulmonary trunk was measured at 1000× magnification.

Statistical analysis

The data were analysed using descriptive and inferential statistics. All values are presented as mean ± standard error of mean (SEM) for ten rats in each of the two groups of rats. The significance of difference in the means of all parameters reported for the two groups of animals was determined using paired sample student t-test and a p-value of < 0.05 (two tailed) was considered as significant.

RESULTS

Physical observation

Prior to STZ administration, there was no significant difference in the average weights of the control and diabetic groups of rats. By the end of the first week after diabetes mellitus was experimentally induced, the weights of the diabetic rats were significantly reduced despite the increase in food and fluid intake in these animals. This weight loss continued for four weeks after STZ administration (Fig. 1). At the end of the experimental period there was a significant decrease in the body weights of the diabetic rats when compared to the control rats (Table 1). The animals manifested alopecia (loss of hair on the head) (Fig. 2) and polyurea shown by marked wetness of the ventral body surface of the animals.

Changes in blood glucose level

Prior to STZ administration, the fasting blood glucose levels did not differ significantly between

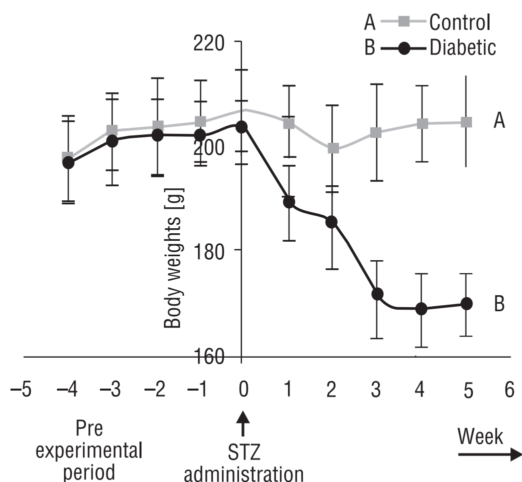

Figure 1. Weekly changes in the body weights of control and diabetic rats.

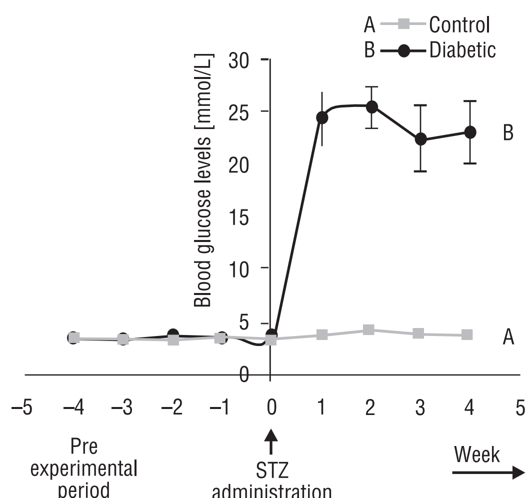

Figure 3. Weekly changes in the blood glucose levels of control and diabetic rats.

Table 1. Mean values of the body weight and blood glucose level of control and diabetic rats after streptozotocin administration

Group	Body weight [g]	Blood glucose level [mmol/L]
Group A (initial)	206.64 ± 9.805[a]	3.59 ± 0.150[a]
Group A + citrate buffer	204.28 ± 8.307[a]	3.66 ± 0.200[a]
Group B (initial)	202.26 ± 6.856[a]	3.33 ± 0.176[a]
Group B + streptozotocin	171.28 ± 5.143[b]	24.17 ± 2.555[b]

a, b within column signifies that means with different letters differs significantly at $p < 0.05$ (two tailed T-test) while means with the same letters does not differ significantly at $p < 0.05$ (two tailed T-test)

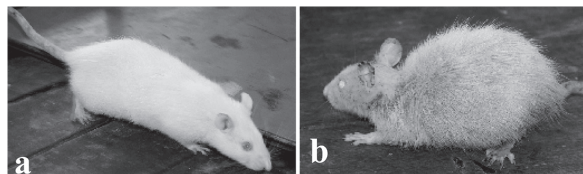

Figure 2. Photographs of control (**A**) and diabetic (**B**) rats.

the control and diabetic groups of rats. The blood glucose levels gradually increased during the five-day period of STZ administration. One week after administration of STZ, the blood glucose level was significantly higher in the group-B rats. The blood glucose level of these rats remained elevated over a period of four weeks (Fig. 3). Control rats treated with citrate buffer maintained a normal blood glucose level throughout the period of the experiment.

Changes in weight of left ventricle aorta and pulmonary trunk

The mean weights of the heart and LV were significantly higher in the control rats when compared with STZ treated rats. In addition, the relative ratio of the left ventricle to the weight of the heart expressed as a percentage was significantly higher in the control group of rats when compared to the diabetic rats. However, there was no significant difference in the weights of the aorta and pulmonary trunk of the control and the diabetic groups of rats (Table 2).

Table 2. Mean value of weights of the left ventricle, aorta and pulmonary trunk in control and diabetic rats

	Wet heart weight [g]	Wet LV weight [g]	Relative heart weight (%)	$\frac{LVW}{HW} \times 100$ (%)	Aorta weight [g]	Pulmonary trunk weight [g]
Control	0.8723 ± 0.0378	0.2624 ± 0.0341	0.3437 ± 0.0204	29.6835 ± 3.3140	0.1270 ± 0.0383	0.0218 ± 0.0020
Diabetic	0.6053 ± 0.0382*	0.1302 ± 0.0109*	0.4229 ± 0.0270*	21.8229 ± 1.9166*	0.1162 ± 0.0126	0.0172 ± 0.0084

*Values are significantly different from control at $p < 0.05$ (two tailed T-test); LV — left verticle; LVW — weight of the left verticle; HW — weight of the heart

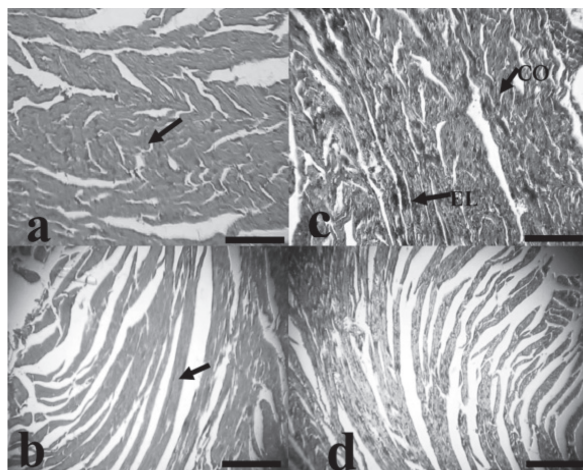

Figure 4. Transverse section of the left ventricle of the control and diabetic rats stained with H & E (**A** and **B**, respectively) and Verhoeff-van Gieson stain (**C** and **D**, respectively). Note the distribution of the cardiac muscle cells in **A** and **B** (black arrow); note also the distribution of elastic (EL) and collagen (CO) fibres in **C** and **D**. Bar = 9.09 μm.

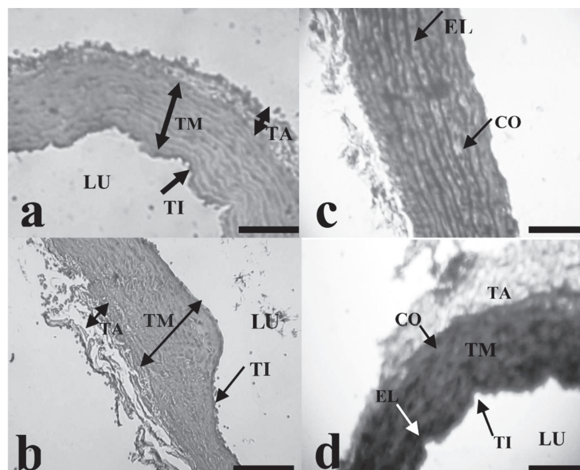

Figure 5. Transverse section of the aorta of the control and diabetic rats stained with H & E (**A** and **B**, respectively) and Verhoeff--van Gieson stain (**C** and **D**, respectively). Note the tunica intima (TI), tunica media (TM), and tunica adventitia (TA) of the wall of aorta in **A** and **B**; note also the distribution of elastic (EL) and collagen (CO) fibres in **C** and **D**; LU — lumen; Bar = 9.09 μm.

Morphological observations

Histological observation of the LV, aorta, and pulmonary trunk in the two experimental groups revealed that there was an alteration in the cytoarchitecture of the STZ treated sections as compared with the control. The myocardium in the control section presented well-organized myofibrils with long cylindrical cells with a centrally placed nucleus as opposed to the derangement of myofibrils and scarce cellular components observed in the STZ treated sections. Distribution of elastic and collagen fibres in the wall of the aorta, pulmonary trunk, and LV was very scarce in the diabetic group when compared to the control group. The distribution of elastic (EL) and collagen (CO) fibres in the wall of the heart and blood vessels was evident by the high staining intensities of Verhoeff stain and van-Gieson's stain, as shown in Figures 4, 5, and 6. The tunica intima of the aorta and pulmonary trunk were significantly thicker in the diabetic rats than in normal rats.

Morphometric analysis

The results of the morphometric analysis revealed that the luminal diameter of the LV, aorta, and pulmonary trunk were significantly reduced in the diabetic group when compared to the control group, and the wall of these cardiovascular structures was significantly thicker in the diabetic group than in the control group (Table 3). The tunica intima of the aorta and pulmonary trunk were significantly thicker in the diabetic group than in the control group (Table 4). This signifies gradual deposition of atherosclerotic plaques in the intima of these vessels. However, there was no significant difference between the tunica media and adventitia of the control rats and that of diabetic rats.

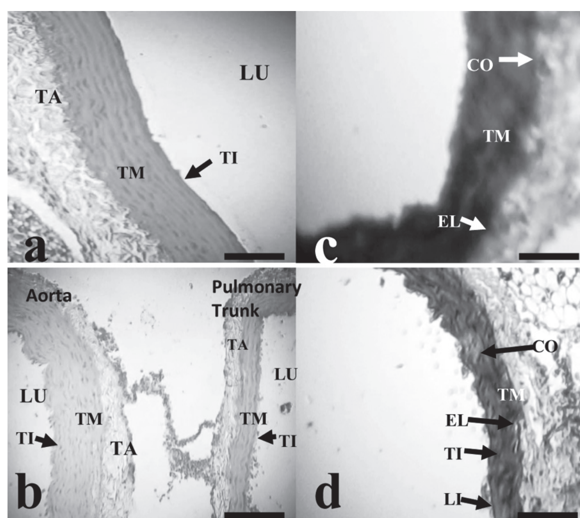

Figure 6. Transverse section of the pulmonary trunk of the control and diabetic rats stained with H & E (**A** and **B**, respectively) and Verhoeff-van Gieson stain (**C** and **D**, respectively). Note the tunica intima (TI), tunica media (TM), and tunica adventitia (TA) of the wall of the pulmonary trunk in **A** and **B**; note also the distribution of elastic (EL) and collagen (CO) fibres in **C** and **D**. There is evidence of lipid deposits (LI) in the tunica intima of the diabetic rats, as shown in **D**; LU — lumen; Bar = 9.09 μm.

Table 3. Lumen diameter, wall thickness and wall to lumen ratio of left ventricle (LV), aorta and pulmonary trunk (PT) in control and diabetic rats

	LV wall thickness [μm]	LV lumen diameter [μm]	LV wall: lumen ratio	Aorta wall thickness [μm]	Aorta lumen diameter [μm]	Aorta wall: lumen ratio	PT wall thickness [μm]	PT lumen diameter [μm]	PT wall: lumen ratio
Control	529.10 ± ± 28.096	2841.23 ± ± 104.44	0.1875 ± ± 0.013	151.75 ± ± 9.765	1192.53 ± ± 55.341	0.129 ± ± 0.009	63.35 ± ± 5.415	749.26 ± ± 15.316	0.085 ± ± 0.007
Diabetic	692.96 ± ± 20.768*	1910.18 ± ± 45.06*	0.3637 ± ± 0.011*	217.58 ± ± 12.773*	928.33 ± ± 17.860*	0.234 ± ± 0.012*	97.61 ± ± 4.738*	555.88 ± ± 10.250*	0.175 ± ± 0.007*

*Values are significantly different from control at $p < 0.05$ (two tailed T-test)

Table 4. Thicknesses of the tunica intima, media and adventitia of the aorta and pulmonary trunk in control and diabetic rats

	Thickness of aorta intima [μm]	Thickness of aorta media [μm]	Thickness of adventitia [μm]	Thickness of pulmonary trunk intima [μm]	Thickness of pulmonary trunk media [μm]	Thickness of pulmonary trunk adventitia [μm]
Control	3.62 ± 0.353	96.52 ± 11.501	45.52 ± 3.840	3.22 ± 0.244	45.15 ± 2.602	19.80 ± 1.222
Diabetes	14.59 ± 1.189*	104.90 ± 9.050	41.280 ± 2.595	11.307 ± 0.863*	40.16 ± 2.628	17.16 ± 0.719

*Values are significantly different from control at $p < 0.05$ (two tailed T-test)

DISCUSSION

Despite the dramatic improvements in diagnostic procedures and treatment of diabetic patients, cardiovascular complications are still implicated in the death of these patients [5]. Consequently, the study of heart disease in diabetic patients is of great clinical importance. Dyslipidaemia, a primary risk factor for the development of atherosclerosis in diabetic patients, has been reported to impair aortic elastic properties (aortic distensibility), which is an important determinant of left ventricular function and coronary blood flow [29]. The histopathologic and morphometric results of this study show that the tunica intima of the aorta and pulmonary trunk are significantly thicker in diabetic rats than in normal rats. This may be due to deposition of lipid droplets in these vessels to form atherosclerotic plaques. The distribution of elastic and collagen fibres in the wall of the aorta, pulmonary trunk, and left ventricle is very scarce in the diabetic rats when compared to normal rats, leading to a reduction in the elasticity of these blood vessels. This, together with the reduction in the luminal diameter of these vessels, may lead to hypertension in these animals. Some of the studies in the literature show that the left ventricle is seriously affected by diabetes, whereas some of the others demonstrate that the RV is more severely affected. In most previous studies, the RV was not even evaluated, partly because only the left ventricle was subject to the primary haemodynamic overload, and the pathological processes were expected primarily to involve the left ventricle. Study of the mechanisms of biventricular pathology may provide insight into the pathogenesis of ventricular damage and subsequent heart failure. Diabetes has been shown to alter the activities of antioxidant enzymes in the heart [15, 19, 31, 32], which is a well-known implication of increased ROS generation. Researchers have reported increased angiotensin II levels in diabetic hearts [11, 23, 30]. An important result of elevated angiotensin II is promoted activation of NADPH-oxidase, which is a major source of ROS [4, 7, 14, 22]. G6PD and 6PGD activities were found to be increased in diabetic hearts, and these are probably due to increased NADPH production, a substrate of NADPH oxidases. The activities of antioxidant enzymes GR, GSH-Px, and CAT also increased in diabetic hearts, and AT1 receptor blocker candesartan-cilexetil normalized their activities significantly. Recent findings [34] that showed significant reduction of TBARS levels in diabetic hearts after candesartan treatment support this proposal and, consequently, our hypothesis, which attributes a protective role to AT1 receptor blockage against oxidative stress induced by diabetes.

Our findings suggest that diebetes mellitus causes some alterations that may contribute to the development of cardiac and cardiovascular disease in diabetes. There is a reduction in the elastic and collagen fibres in diabetic hearts (especially the left ventricle),

aorta, and pulmonary trunk. In previously published papers [8, 21], the effect of diabetes on the hearts of animals and/or humans that were diabetic for long periods of time (mostly from 2 months to 10 years) have been investigated [24, 25]. The present study demonstrates that even a relatively short term (4 weeks) of diabetes induces significant effects, as mentioned above, on the left ventricular myocardium, aorta, and pulmonary trunk in rats.

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