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COMPARISON OF EFFECTS OF ATENOLOL, CARVEDILOL AND NEBIVOLOL ON STREPTOZOTOCIN INDUCED DIABETES ASSOCIATED WITH CARDIOVASCULAR COMPLICATIONS

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Abstract

The present study was carried out to study the effect of atenolol, carvediloland nebivolol on cardiovascular complications associated with type 1 diabetes mellitus in rats. Single i.p. Injection of 60 mg/kg streptozotocin (STZ) produced type 1 diabetes in male wistar rats.Atenolol (10mg/kg/day), carvedilol (10mg/kg/day) and nebivolol (2mg/kg/day) were administered for 8 weeks after which various biochemical and cardiac parameters were measured. STZ produced hyperglycemia, dyslipidemia, hypertension, bradycardia and cardiac hypertrophy. Chronic treatment with carvedilol and nebivolol produced significant reduction in glucose levels, improvement in oxidative stressand does not altered serum triglyceride, cholesterol and HDL levels. Atenolol,significantly increasedglucose, cholesterol and triglyceride levels and does not improve oxidative stress. Blood pressure and other cardiovascular parameter were controlled by all the drug treatment. In conclusion, Nebivolol and Carvedilol significantlyimproved glycemic control and oxidative stress in animals with diabetes mellitus associated cardiovascular complications and this improvement was significantly better than that obtained with atenolol. Nebivolol and Carvedilol have no dismetabolic effects and they have reduced the incidence of newly diagnosed diabetes compared to atenolol. Carvedilol and nebivololappears to be beneficial agents as compared to atenolol.Carvedilol and nebivolol is the ideal beta-blocker for the patient with diabetes and associated cardiovascular complications.

INTRODUCTION

Hypertension is an extremely common comorbid condition in diabetes affecting approximately 20-60% of patients with diabetes, depending on obesity, ethnicity and age. [1] Hypertension is approximately twice as frequent in patients with diabetes as compared with patients without the disease. [2] To attain the current guideline recommendations for controlling of blood pressure (BP) in persons with DM(130/80 mm Hg), use of several antihypertensive agents is required.[3] All guidelines recommend beta-blockers among other classes to achieve this goal. [4, 5] Beta-blockers have been shown to decrease cardiac events in the diabetic patient with known coronary artery disease. [6, 7] However, beta-blockers increase insulin resistance so that in the non-diabetic hypertensive subject the risk of developing diabetes increases from 25 to 30%. [8,9] Because of the worsening of insulin resistance, beta-blockers not only led to an increased risk of developing diabetes and worsening of glycaemic control with pre-existing diabetes but also led to an increase in cardiac risk factors (lower HDL, higher triglycerides and an increased proportion of small dense highly atherogenic LDL particles). Because of the consequences of the increase in insulin resistance, physicians are reluctant to prescribe beta-blockers either to insulin resistant or to diabetic patients. [10]

Atenolol is a second generation hydrophilic beta 1- blocker. Nebivolol is a selective third generation beta 1 lipophilic blocker and devoid of intrinsic sympathomimetic activity. It also modulates NO release. Carvedilol, a non selective third generation beta-blocker and selective alpha-1-adrenoreceptor blocking activity with a beta to alpha-1 ratio of 7.6 : 1 for the 50 mg dose [11, 12]. Furthermore, by its effects on lipid peroxidation, carvedilol prevents depletion of endogenous antioxidants and improves endothelial function by relieving oxidative stress [13]. Either through its anti-inflammatory effect or through its alpha-1-adrenoreceptor- induced vasodilating effect, carvedilol reduces insulin resistance, whereas traditional vasoconstricting beta blockers have the opposite effect [14]. Hence, in present investigation we have carried out comparative evaluation of atenolol, carvedilol and nebivolol on metabolic parameter, antioxidant and

cardiovascular complications associated with STZ-induced type 1 diabetic rats.

Materials and methods

Chemicals

STZ was purchased from Sigma-Aldrich (USA), and the glucose GOD-POD kit, as well as the triglyceride, total cholesterol, cholesterol-high density lipoprotein (HDL), lactate dehydrogenase (LDH) and the CK-MB kits were purchased from Span Diagnostics Ltd (India). Other chemicals used were of analytical reagent grade.

Animals

Male Wistar rats weighing 250 g to 300 g were obtained from the animal facility of the Zydus Research Centre (India). They were maintained under standard environmental conditions (12 h light/dark cycles at 20°C to 25°C, and controlled humidity) and provided with feed and purified water ad libitum. All experiments and protocols described in the present study were approved by the Institution of Animal Ethics Committee, and are in accordance with guidelines as per the Guide forthe Care and Use of Laboratory Animals.Permission was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals. (CPCSEA)

Induction of diabetes and treatment protocol

Diabetes was induced by a single-intraperitoneal injection of STZ (60 mg/kg i.p) dissolved in citrate buffer. The control animals were injected with equal volumes of the vehicle. Forty eight hours after STZ injection, animals showing blood glucose levels (>250 mg/dl) were considered to be diabetic. Animals were divided into five groups of six each: normal control, diabetic control, diabetic animals treated with atenolol (10 mg/kg/orally/day), carvedilol (10 mg/kg/orally/day) and nebivolol respectively (2 mg/kg/orally/day). Treatment was started seven days after STZ injection, and was given daily for eight weeks. Food intake, water intake and body weight gain were measured. Blood pressures and heart rate were measured at the end of the eight-week treatment period.

At the end of the eight-week treatment period, the animals were kept on an overnight fast, and the blood samples were

collected. The blood was allowed to clot for 30 min at room temperature and then centrifuged at 5000 rpm for 20 min. The serum was separated and stored at –20° C until analysis was complete. Serum samples were analyzed spectrometrically (Shimadzu UV-1601, Japan) for serum glucose, triglyceride, total cholesterol, HDL-cholesterol, LDH and CK-MB using their respective kits. Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated from the total cholesterol, triglyceride and HDL values.

Isolated heart experiment

The animals were euthanized and the hearts were isolated and mounted as per the Langendorff heart technique. The hearts were perfused with Chenoweth Koelle buffer (119.8 mmol/L NaCl, 5.6 mmol/L KCl, 2.88 mmol/L CaCl2, 4.5 mmol/L MgCl2, 3.8 mmol/L NaHCO3 and 5 mmol/L glucose), and were continuously infused with 95% O2 and 5% CO2 carbogen. The blood pressure and heart rate were recorded using a transducer attached to the students' physiographs. At the end of the study, the hearts were blotted with filter paper to remove excess water and the remaining extraneous tissues were removed. The weight of the hearts were noted to calculate the index of hypertrophy as wet left ventricular weight to body weight (LV/BW) and heart weight to body weight (HW/BW), and were subjected to assessment of antioxidant parameters and estimation of LV collagen and protein content.

Assessment of oxidative stress-related markers in the heart

Heart tissues were finely sliced and homogenized in chilled tris buffer. The homogenates were centrifuged and the clear supernant was used for estimation of various antioxidant parameters such as superoxide dismutase (SOD), catalase, lipid peroxidation or malondialdehyde (MDA), and reduced glutathione (GSH). SOD was determined by the Misra and Fridovich [15]method, catalase was determined by the Aebi [16] method, and GSH was determined according to the method described by Moron et al [17] MDA formation was determined using the Slater and Sawyer [18] method. Antioxidant activity in the liver was expressed in terms

of protein content, which was measured as per the method reported by Lowry et al[19]

Statistical analysis

Values were expressed as mean ± SEM. The results were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered to be statistically significant.

Results

Effect on biochemical parameters

Diabetic rats were found to exhibit significant (P<0.05) hyperglycemia, hypertriglyceridemia and hypercholesteremia as compared to control rats. Treatment with atenolol produced significant increase in elevated serum glucose, triglycerides, and cholesterollevels. whereas, treatment with carvedilol and nebivolol significantly reduced elevated glucose levels (Table 1). However, carvedilol and nebivolol treatment not altered the elevated triglycerides, cholesterol, LDL and VLDL levels of diabetic rats.

Effect on hemodynamic parameters

Mean blood pressure was found to be increased, and heart rate were found to be decreased in diabetic rats compared with the control animals. Chronic treatment with atenolol, carvedilol and nebivolol showed a significant (P<0.05) increase in heart rate and a decrease in blood pressure compared with diabetic control animals (Fig 1 A, 1 B).

Cardiac hypertrophy index

The ratio of wet LV/BW and HW/BW served as an index of cardiac hypertrophy. The LV/BW, HW/BW, LV collagen and protein content were found to be significantly increased in diabetic rats. Treatment with atenolol, carvedilol and nebivolol significantly (P<0.05) decreased the LV/ BW, HW/BW ratio, left ventricular collagen and protein content (Fig 2-A, 2-B, 2-C and 2-D).

Effect on serum LDH and CK-MB

Diabetic rats showed a significant increase in serum CK-MB activity compared with control rats. Administration of atenolol, carvedilol and nebivolol significantly decreased the diabetes-induced increase in serum CK-MB activity in diabetic animals. Serum LDH levels of diabetic animals were also significantly increased compared with

control animals. Administration of atenolol, carvedilol and nebivolol to diabetic rats significantly (P<0.05) attenuated LDH serum activity level compared with diabetic control animals (Figure 2-E, 2-F).

Effect on antioxidant parameters in the heart

STZ diabetic rats were found to have decreased SOD, GSH and catalase enzyme levels in the heart compared with control rats. Treatment with carvedilol and nebivolol produced a significant increase in these enzyme levels whereas treatment with atenolol did not change in enzyme levels. STZ diabetic rats were also found to exhibit a significant increase in MDA levels in the heart compared with control rats. Treatment with carvedilol and atenolol produced significant (P<0.05) decreases in MDA levels whereas treatment with atenolol did not affect MDA levels (Table 2).

Discussion

In the present investigation STZ produced cardinal signs and characteristics of diabetes viz polyphagia, polyuria, polydipsia, hyperglycemia, hypoinsulinemia, dyslipidemia and cardiovascular alterations like bradycardia, hypertension and hypertrophy of heart which are consistent with those reported earlier [20]

Intravenous injection of STZ produces fragmentation of DNA of β-cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia [21]In present study, STZ produced a significant increase in glucose levels type 1 diabetic rats. Treatment with nebivololand carvedilol significantly reduced the serum glucose levels. Thus, reduction in the serum glucose levels may be attributed to increase in glucose uptake or enhancing insulin sensitivity. However, further studies are required to be carried out to find out precise mechanism of action. Whereastreatments with atenolol significantly increase glucose level. These results show that nebivolol and carvedilol possesses a beneficial effect over atenolol on the glycemic status of diabetic hypertensive rats. Further investigations are required to elaborate the mechanism underlying this effect of nebivolol and carvedilol.

It has been reported that in STZ-diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia [22] A low level of plasma high-density lipoprotein cholesterol (HDL-C) is one component of a cluster of coronary disease risk factors that also includes abdominal obesity, hypertension, hyperinsulinemia, and insulin resistance [23] In the present investigation serum cholesterol and triglyceride levels of diabetic rats were found to be significantly increase by the treatment with atenolol. Extensive trials suggest that non selective beta blockers without intrinsic sympathomimetic activity increase serum triglyceride levels and decrease high densitylipoprotein levels [24].Several studies have suggested that carbohydrate and lipid metabolic abnormalities, such as hyperglycemia and hyperlipidemia, maycontribute to the development of cardiac dysfunction in diabetes mellitus [25] .Whereas treatments with nebivolol and carvedilol does not alter the triglyceride, cholesterol and HDL levels.

Increase in blood pressure after treatment with STZ has been reported by several workers. In our study also, blood pressure of STZ-diabetic animals was found to be significantly higher as compared to non-diabetic animals. Atenolol, carvedilol and nebivolol prevented the rise in blood pressure in diabetic animals.Bradycardia is frequently observed in STZ diabetic rats [26]. In our study, also heart rate of STZ-diabetic animals was found to be significantly lower in diabetic rats as compared to non-diabetic. Atenolol, carvedilol and nebivolol prevented the rise in blood pressure in diabetic animals.Beta blockers, especially lipophilic ones, are reported to up regulate the cardiac β1 receptors and also inhibit the stimulatory auto β1 receptor auto antibodies. This may be a possible mechanism responsible for the beneficial effect in diabetes induced cardiac dysfunction.

Data from Framingham study indicate that, left ventricular hypertrophy (LVH) is not a benign compensatory process but an independent risk factor for congestive heart failure, coronary artery disease, and sudden death. In the presence of diabetes, in hypertensive subjects, damage to the myocardium due to hypertension appears to be accelerated. Diabetic hypertensive patients have greater interventricular

septum and posterior wall thickness than non-diabetic hypertensive patients. Consequently, left ventricular mass index may be greater in patients with hypertension and diabetes mellitus than in those without diabetes mellitus. [27] In the present study, the wet heart weight to body weight ratio, an index of cardiac hypertrophy, was found to be increased in diabetic hearts. Atenolol, carvedilol and nebivolol treatment significantly reduced the cardiac hypertrophy and left ventricular hypertrophy in treated animals.

Serum LDH and CK-MB activities were found to be increased in STZ diabetic rats, possibly due to myocardial dysfunction because ithas been previously reported that serum LDH and CK-MB activitieswere found to be increased in cardiomyopathy [28] Serum CK-MB and LDH levels were also reported to increase in diabetic patients, andmay serve as a marker for cardiovascular risk and cardiac musculardamage [29] . In the present study, there was a significant decrease inserum LDH and CK-MB levels observed with treatment of atenolol,carvedilol and nebivolol indicating good cardioprotection.

Increased reactive oxygen species (ROS) production in the diabetic heart is a contributing factor in the development and progression of diabetic cardiomyopathy. Increased ROS amplifieshyperglycemia-induced activation of protein kinase C isoforms, increased formation of glucose derived advanced glycation end products,and glucose flux through the aldose reductasepathways [30]. All of these factors may contribute to the development of cardiac complications in diabetes mellitus. Increased ROS generation mayactivate maladaptivesignalling pathways, which may lead to celldeath and could promote abnormal cardiac remodelling, which ultimately may contribute to the characteristic morphological and functionalabnormalities that are associated with diabetic cardiomyopathy.Thus, the levels of antioxidant enzymes, such as GSH, SOD or catalase,are decreased in the diabetic heart. The strategies either to reduce ROS or augment myocardial antioxidant defense mechanisms mighthave therapeutic efficacy in improving myocardial function in diabetesmellitus. Treatment with carvedilol and nebivololincreased the levels

ofendogenous antioxidants and decreased lipid peroxidation in diabetic rats. In our study, anincrease in antioxidant enzyme levels in the heart were observed after treatment with carvedilol and nebivolol in diabetic animals, which can reverse diabeticcardiomyopathy. Thus, diabetics patients who are at an increasedrisk of cardiac dysfunction may benefit both from an improvement in glucose as well as from the antioxidant and cardioprotectiveeffect of carvedilol and nebivolol.

Conclusions

Atenolol, Nebivolol and Carvedilol significantly prevents diabetes induced cardiovascular complications by significantly improving CK-MB, LDH, cardiac hypertrophy index, Collagen, left ventricular hypertrophy index and blood pressure compared with diabetic group. Nebivolol and Carvedilol significantlyimproved glycemic control and oxidative stress in animals with diabetes mellitus associated cardiovascular complications and this improvement was significantly better than that obtained with atenolol. Nebivolol and Carvedilol have no dismetabolic effects and they have reduced the incidence of newly diagnosed diabetes compared to atenolol. Carvedilol and nebivololappears to be beneficial agents as compared to atenolol.

Carvedilol and nebivolol, nonselective beta-blockers with vasodilating properties, is the ideal beta-blocker for the patient with diabetes and associated cardiovascular complications.

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Table 1

Effects of atenolol, carvedilol and nebivolol on Glucose and Lipid Profile in diabetes induced cardiovascular complications

Parameters (mg/dl)	NC	DC	DCA	DCC	DCN
Glucose	103.00±6.92	354.67*±10.75	392.33$^{\#}$±10.37	335.83$^{\#}$±8.48	321.67$^{\#}$±6.89
Cholesterol	93.50±3.68	168.17*±7.37	198.00$^{\#}$±8.03	160.33±4.68	173.67±3.20
Triglyceride	75.83±4.88	129.83*±6.04	160.83$^{\#}$±6.97	127.67±4.60	123.67±5.41
HDL cholesterol	46.00±3.11	31.00*±1.91	42.83±2.68	34.83±2.09	34.50±2.86
LDL cholesterol	32.33±2.01	111.20*±8.43	123.00±8.91	99.97±5.40	114.43±2.95
VLDL cholesterol	15.17±0.98	25.97*±1.21	32.17±1.39	25.53±0.92	24.73±1.08

Data presented as mean±SEM *significantly different from diabetic control (P<0.05)

Table 2

Effect of atenolol, carvedilol and nebivolol on antioxidant parameters in diabetes induced cardiovascular complications

Parameters (mg/dl)	NC	DC	DCA	DCC	DCN
MDH(nmoles/mg protein)	3.85±0.25	8.05*±0.66	7.89±0.54	4.93$^{\#}$±0.46	4.55$^{\#}$±0.42
Glutathione(µg/mg protein)	8.91±0.48	2.33*±0.26	2.37±0.30	6.24$^{\#}$±0.44	6.99$^{\#}$±0.51
SOD(U/min/mg protein)	2.23±0.25	0.90*±.17	0.98±0.20	2.04$^{\#}$±0.29	2.18$^{\#}$±0.13
Catalase(U/min/mg protein)	6.23±0.45	2.38*±0.35	2.54±0.49	4.81$^{\#}$±0..34	5.06$^{\#}$±0.22

Data presented as mean±SEM *significantly different from diabetic control (P<0.05)

MDA: melondialdehyde, SOD: superoxide dismutase

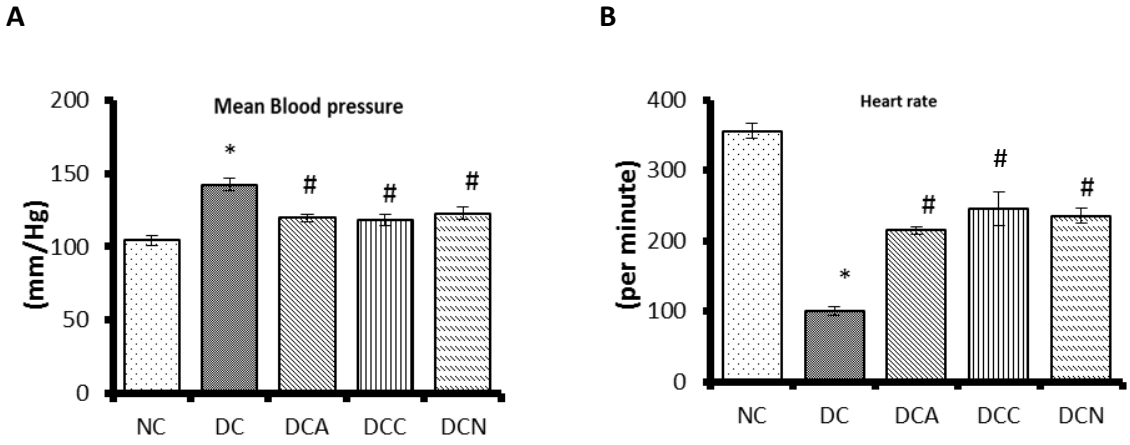
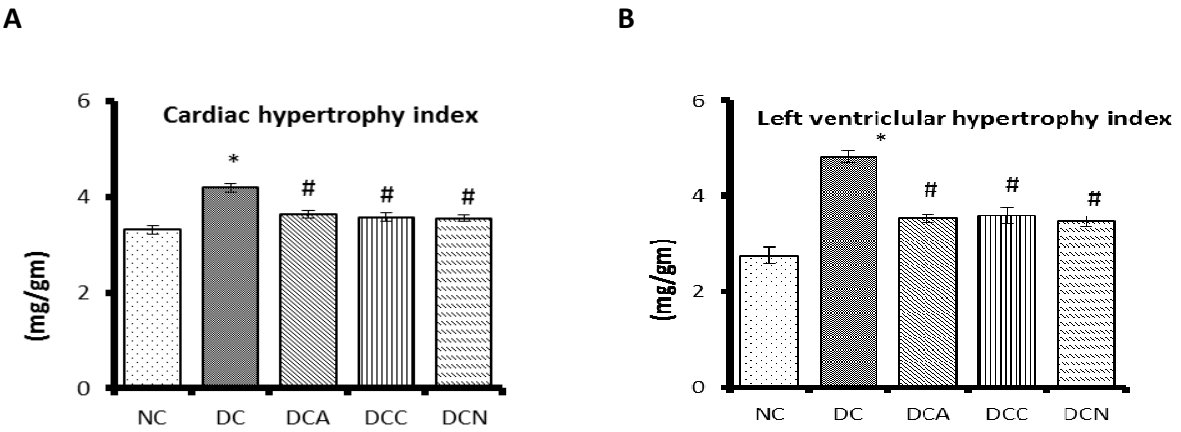


Fig: 1 Effects of atenolol, carvedilol and nebivolol on hemodynamic parameters. Each bar represents mean±SEM of six animals NC= normal control, DC= diabetic control, DCA= diabetic rats treated with atenolol, DCC= diabetic rats treated with carvedilol, DCN= diabetic rats treated with nebivolol * significantly different from diabetic rats (P<0.05)



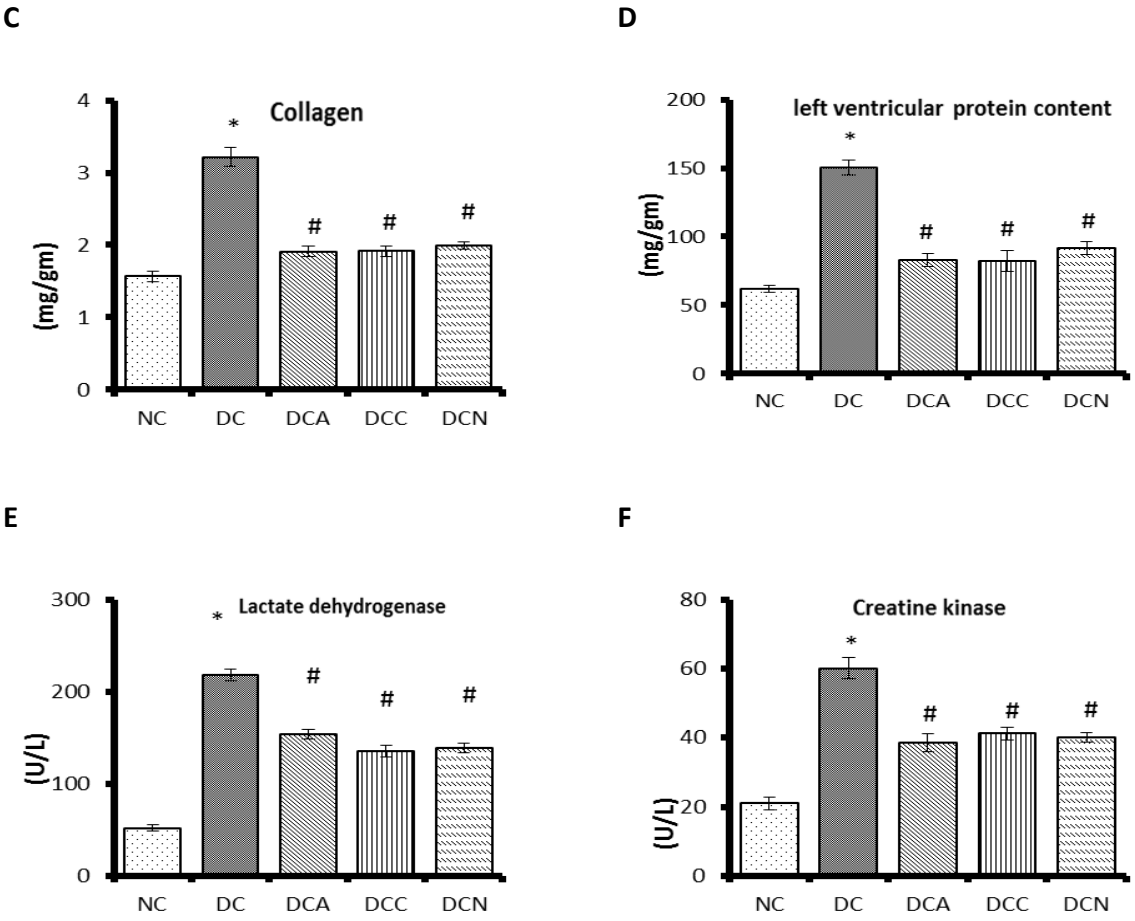


Fig: 2 Effects of atenolol, carvedilol and nebivolol on cardiovascular parameters. Each bar represents mean±SEM of six animals NC= normal control, DC= diabetic control, DCA= diabetic rats treated with atenolol, DCC= diabetic rats treated with carvedilol, DCN= diabetic rats treated with nebivolol * significantly different from diabetic rats (P<0.05)

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