"COMPARATIVE EVALUATION OF CARVEDILOL, METOPROLOL AND ATENOLOL ON CARDIOVASCULAR COMPLICATIONS ASSOCIATED WITH EXPERIMENTAL TYPE 2 DIABETES"

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BY

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CERTIFICATE

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DECLARATION

I declare that the thesis "Comparative evaluation of carvedilol, metoprolol and Atenolol On cardiovascular complications associated with experimental type 2 diabetes" has been prepared by me under the guidance of Dr. Shital Panchal, Assistant Professor, Dr. Bhoomika Goyal, Assistant Professor, Ms. Shraddha Bhadada, Assistant Professor Department of Pharmacology, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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Science is facts; just as houses are made of stones, so is science made of facts; but a pile of stones are not a house and a collection of facts is not necessarily science.

*There is a famous saying "**Work always Works**". There is always the danger that we may just do the work for the sake of the work. This is where the respect, love and the devotion come in - that we do it to God, to Christ and that's why we try to do it as beautifully as possible.*

"God is like a big circle, whose centre is Everywhere but circumference is nowhere"

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1. ABSTRACT

Objective- Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with type 2 diabetes. β- receptor blockers provide cardiovascular protection in diabetic patients. We have studied the effect of 8 weeks treatment with carvedilol, metoprolol and atenolol on cardiovascular complications associated with streptozotocin (STZ) induced diabetes in neonatal rats.

Materials and methods- Wistar rats of 5 day age were made diabetic with STZ (80 mg kg-1, ip). Various biochemical, cardiac parameters and hemodynamic parameters were measured at the end of 8 weeks of treatment with carvedilol (1mg/kg/day, p.o.), metoprolol (10mg/kg/day, p.o) and atenolol (10mg/kg/day, p.o) like general parameters (body weight, food intake and water intake), glucose and insulin levels, cholesterol, triglyceride, very low density lipoproteins, atherogenic index and high density lipids, creatinine kinase and lactate dehydrogenase enzyme and C-reactive protein (CRP) levels, heart rate, blood pressure, rate of pressure development and decay, cardiac hypertrophy and antioxidant levels.

Results- STZ produced hyperglycemia, hyperinsulinemia, hyperlipidemia, increased blood presser, increased creatinine kinase and lactate dehydrogenase enzymes and C-Reactive protein (CRP) levels, reduction in heart rate, blood presser, cardiac hypertrophy. Chronic treatment with carvedilol and metoprolol significantly ($P<0.05$) prevented STZ induced hypertension, hyperglycemia and hyperinsulinemia. Atenolol did not produce reduction in hyperglycemia and hyperinsulinemia. Carvedilol and metoprolol significantly ($P<0.05$) reduced the elevated cholesterol, very low density lipoprotein (VLDL), atherogenic index and triglyceride levels in diabetic rats and increased the lower high density lipoprotein (HDL)-cholesterol levels while atenolol showed no significant effect on lipid profile. Further, all drugs produced a significant ($P<0.05$) reduction in the elevated levels of CRP and other cardiac enzyme markers like Lactate de-hydrogenase and creatinine kinase of diabetic rats. STZ-induced bradycardia was also prevented by carvedilol and metoprolol treatment and atenolol showed no significant ($P<0.05$) effect on heart rate. All three drugs produced beneficial effect by preventing cardiac hypertrophy as evident from cardiac hypertrophy index and left ventricular hypertrophy.

Carvedilol prevent STZ induced oxidative stress but metoprolol and atenolol showed no significant ($P<0.05$) effect on antioxidant levels.

Conclusion- Our data suggests that carvedilol and metoprolol have beneficial effect in cardiovascular complications associated with streptozotocin (STZ) induced diabetes in neonatal rats as depicted by prevention of hyperglycemia, hyperinsulinaemia, hyperlipidemia, hypertension, bradycardia, cardiac and left ventricular hypertrophy and reduction in cardiac biomarker levels. Further, carvedilol appears to be more beneficial than metoprolol since it also prevents oxidative stress. However, atenolol shows no beneficial effect on diabetes induced cardiovascular complications.

Keywords: carvedilol, metoprolol, atenolol, streptozotocin, diabetes, cardiovascular complications

2. INTRODUCTION

Systemic hypertension is a serious adverse consequence of diabetes mellitus, contributing appreciably to cardiovascular morbidity and mortality by the acceleration of diabetic micro vascular and macro vascular complications. Hypertension is very frequently associated with diabetic subjects (The Hypertension in Diabetes Study Group, 1993), i.e., about 50% of diabetics, irrespective of whether they are Type 1 or Type 2, are hypertensive. High blood pressure (BP), obesity, and abnormal lipid profile, which often coexist with diabetes, tend to be associated with preclinical cardiovascular abnormalities and may contribute to the association of diabetes with cardiovascular events. (Gu et al., 1999)

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with diabetes. Indeed, although there have been substantial reductions in morbidity and mortality related to CVD in patients without diabetes over the past 10 years, it seems that such improvements have not been seen in patients with diabetes. Epidemiological data have shown that blood glucose levels are related to CVD, and so achieving normoglycaemia would be expected to reduce the incidence of cardiovascular events. However, several large prospective trials (EDIC, ADVANCE, UKPDS, VADT, ACCORD) that are powered to detect the effect of intensive glucose control on macrovascular outcomes have provided conflicting results, leading to controversy about the effect of glucose levels on CVD risk. (DeSouza, 2009)

Among patients with type 2 diabetes, coronary heart disease, myocardial infarction stroke, peripheral vascular disease are few important causes of mortality and morbidity among patients with type 2 diabetes. Conventional risk factors contribute similarly to macrovascular complications in patients with type 2 diabetes and nondiabetic subjects, and therefore, other explanations have been sought for enhanced atherothrombosis in type 2 diabetes (UKPDS, 1998). Patients with diabetes are at greater risk for developing microvascular and macrovascular complications, particularly coronary artery disease, kidney disease, cardiomyopathy, heart failure, myocardial infarction, and stroke. Individuals with hypertension in diabetes have

accelerated progression of these complications, leading to exaggerated cardiovascular morbidity and mortality. Management of hypertension in these

patients reduces the risks and progression of diabetes-associated complications. (Hansson et al., 1998)

Several studies have been carried out to study the effect of anti-hypertensive agents on insulin sensitivity, and it has been reported that ACEIs improve insulin sensitivity. Captopril improves insulin sensitivity by augmenting a diminished postprandial forearm blood flow. ACE inhibitors attenuate cardiac myocyte hypertrophy and myocardial fibrosis by raising bradykinin and prostacyclin levels and mediating the release of nitric oxide (Yusuf et al., 2000). A long-acting dihydropyridine calcium-antagonist, such as barnidipine, also improves insulin sensitivity. Calcium channel blockers hyperpolarized the heart and decreased force of contraction which is elevates due to hypertension and cardiac hypertrophy. Diuretics caused, decrease in permeability of the microvasculature, secondary to a reduction in insulin resistance (Fukunaga et al., 2001)..

β-blocker treatment in coronary heart disease is less frequently used in diabetic patients. Only 40–50 % of diabetic patients receive β-blockers after myocardial infarction, a considerably lower number than non-diabetic patients (Löwel et al., 2000). Even in a large recently published intervention study in high risk diabetic patients aiming at reduction of cardiovascular mortality, only 28 % received β-blockers. This occurs paradoxically despite the fact that all large intervention studies have convincingly shown that β-blockers in diabetic patients after AMI are more effective than in non-diabetic patients (HOPE, 2000).

It is reported that selective β1-blocking agents have adverse effects on glucose metabolism, prolong hypoglycaemia or mask hypoglycaemic symptoms and cause diabetic nephropathy. However Sawicki at el. 2001 reported that there are no clear evidences which gives effect of β- blockers on diabetic induced cardiovascular complications. So the role of blockers is unclear in type 2 diabetes.

Clinical trial data of SHEP suggested that 19% of diabetic patients received concomitant atenolol, showed beneficial effect of this class of drugs on the diabetes induced hypertension. (SHEP, 2000). Some experimental study suggested that atenolol had the strongest attenuating effect on the development of hypertensive cardiac hypertrophy in SHR (Asai et al. 2005). Göteborg Metoprolol Trial (GMT) and the Metoprolol In Acute Myocardial Infarction (MIAMI) study, the reduction in mortality and late infarction was even greater than in the non-diabetic groups, with a 58% reduction of diabetic mortality in GMT and a 50% reduction in MIAMI (Malmberg et al., 1989). AVID and COMET trials suggested that carvedilol shown a good results in LV remodeling in CHF patients (Mitchell et al. 2003 and Poole-Wilson et al. 2003).

In experimental and clinical study nonselective β-blokers have been shown to adversely affect serum lipid levels. Although this is less likely to occur with cardioselective β_1-blokers such as atenolol and metoprolol, atenolol nonetheless failed to improve cardiac performance or reduce lipid levels in diabetic patients. Moreover, it caused depression of cardiac function, worsened cardiomyopathy, and caused a number of degenerative changes in the left ventricular tissue of the diabetic heart (Bhadada and Goyal, 2007).

In different clinical trials (GEMINI,COMET), it was found that metoprolol increases insulin resistance and alter glycemic control, whereas carvedilol act as neutral or slightly improve glycemic control. Carvedilol is also improve other parameters of insulin resistance, with the reverse occurring with metoprolol like lipid profile in type 2 diabetes. The above findings regarding second generation beta 1 antagonists and third generation beta antagonists are still insufficient to establish their complete profile of cardiovascular complications induced by type 2 diabetes mellitus. (George et al., 2005).

The clinical studies (GEMINI, COMET) are still ongoing showing the beneficial effects of beta blockers in diabetic patients after myocardial infarction.

Aim of present work:

On the basis of above, the aim of the present study was to investigate the comparative effects of atenolol, metoprolol and carvedilol on the type II diabetes mellitus associated cardiovascular complications, in rats using streptozotocin induced neonatal model of type II diabetes mellitus.

3. REVIEW OF LITERATURE

3.1 DIABETE MELLITUS

Diabetes mellitus is a group of heterogeneous disorders in which carbohydrate metabolism is reduced while that of proteins and lipids is increased. Hyperglycemia is a common end point for all types of DM and is an important parameter to evaluate the efficacy of antidiabetic drugs. As hyperglycemia increase, there is a loss of glucose through urine (glycosuria). Virtually all forms of DM are due to either a decrease in the circulating levels of insulin (insulin deficiency) or a decrease in the response of target tissue to insulin (insulin resistance). Although insulin treatment has largely increased the life expectancy of diabetic patients, diabetes remains the third leading cause of death, the second leading cause of blindness as well as of renal failure. The hallmarks of DM are three "polys": an excessive urine production (polyurea), an excessive thirst (polydipsia) and an excessive eating (polyphagia). The disease has two major forms: type-1 and type-2 DM. recently, two more diabetes disease states have also been added. These are: type 3(other) and type 4 (gestational DM).

3.1.1 Type 1 DM

It is also called "**I**nsulin **D**ependent **D**iabetes **M**ellitus" (IDDM) because in such patent, due to an absolute lack of insulin, regular injections of insulin are needed to save life. Earlier, it was called "juvenile-onset diabetes" because it most commonly develops either before puberty or in youngsters below 20 years of age and persists throughout their life.

IDDM is autoimmune disease of the pancreatic β cells (type – 1A) resulting in their degeneration. It could also be idiopathic (type1B).viral infection such as echovirus can also damage pancreatic β-cells (type 1B). approximately10% of diabetics suffer with type 1 DM. these patient have a low degree of genetic predisposition, yet 15-20% of patient reveal a positive family history and the incidence in homozygous twins is about 50%

Since insulin is not present to aid the entry of glucose in skeletal muscle and body cells, most cells now use fatty acids to produce ATP to compensate and to provide calories. This accelerated fat breakdown generates acetyl CoA. But, due to DM, this acetyl CoA cannot be removed by Kreb's cycle (to H_2O_2 and CO_2) and therefore gets accumulated. In absence of aerobic carbohydrate metabolism, two acetyl CoA molecules joins to form acetoacetic acid, β-hydroxybutyric acid and acetone, which are collectively called ketone bodies. These metabolic products cause metabolic acidosis or diabetic ketoacidosis, which decrease glucose utilization in brain and decreases pH of the blood leading to coma and death. Renal losses of glucose (glycosuria), nitrogenous substances and ketone bodies (ketoacidosis) promote osmotic dieresis (polyuria) that can result in dehydration and thirst (polydipsia).

3.2.2 Type-2 DM

This is also called "**N**on **I**nsulin **D**ependent **D**iabetes **M**ellitus" (NIDDM) or "maturity onset diabetes" (as it occurs late in life). Approximately, 90-95% of diabetics have type-2 diabetes. It usually occurs in people who are over 40 and overweight. Many type 2 diabetics, how ever, have sufficient amount or elevate levels of insulin. For these people diabetes arises not from short relatively insensitive to insulin (peripheral resistance to insulin). Genetic predisposition, in type 2 DM, is important as there is greater than 95% concordance in identical twins.

Classical clinical symptoms of type 2 diabetes are almost same as for type 1, but type 2 diabetics are less prone to develop diabetic ketoacidosis. However, they may develop hyperosmolar coma, a condition characterized by severe hyperglycemia and dehydration. It is a clinical emergency that requires faster management with prompt insulin administration and I.V. fluids.

Various complications develop as a consequence of the metabolic derangements in DM (type-1 and type-2) of ten over many years (particularly if there is poor glycaemic control). As a result there may be **microvascular complications** (e.g, retinopathy, nephropathy and neuropathy) as well as micro vascular complications

such as **atherosclerosis and diabetic dyslipedaemia** (elevated TG_s and LDL). **Diabetic retinopathy and nephropathy** result due to accumulation of Sorbitol produced by glucose reduction by aldose reductase. Sorbitol causes thickening of the basement membrane of capillary endothelium, which leads to the narrowing of the micro vessels and deficiency in tissue perfusions.

3.2 HYPERTENSION AND TYPE 2DIABETES MELLITUS

Systemic hypertension is a serious adverse consequence of diabetes mellitus, contributing appreciably to cardiovascular morbidity and mortality by the acceleration of diabetic microvascular and macrovascular complications (Anderson et al, 1992).

Hypertension is very frequently associated with diabetic subjects (The Hypertension in Diabetes Study Group, 1993), i.e, about 50% of diabetics, irrespective of whether they are Type 1 or Type 2, are hypertensive. Hypertensive diabetics have been reported to have more cardiovascular disease when compared to normotensive diabetics. Total mortality in many epidemiological studies is two to three times higher in hypertensive diabetics when compared to that in normotensive diabetics. In addition, despite decreases in the incidence of heart disease in the general population, the decline is much smaller in people with Type 2 diabetes and may even be rising in women with diabetes (Gu et al, 1999). Following data shows the clinical evidences for association of hypertention with type 2 diabetes

Fig. 3.2.1. Incidence rate of major cardiovascular events in all hypertensive subjects (about n = 19,000) and diabetics (n = 1501) in the hypertension optimal treatment randomized trial (HOT) (Hansson et al, 1998).

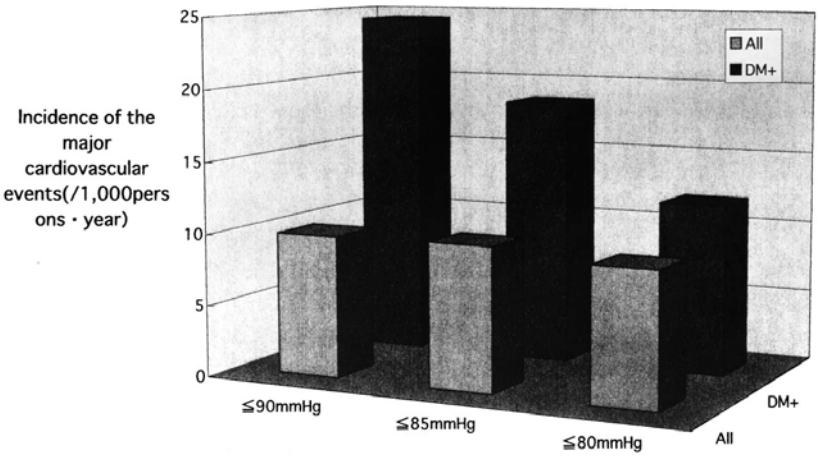


Table 3.2.1 Effect of diabetes on systolic diastolic and Pulse pressure (Vittorio et al, 2001)

Blood Pressure	**Diabetic** , (mm Hg)	**Nondiabetic** (mm Hg)
Systolic BP	137± 24	132± 21
Diastolic BP	72± 11	75± 11
Pulse pressure	65± 18	57± 16

Table 3.2.2 Stratification of risk to quantify prognosis (JSH, 2000)

Risk Factors	**Mild hypertension (140–159/90– 99 mmHg)**	**Moderate hypertension (160– 179/100– 109 mmHg)**	**Severe hypertension (180/110 mmHg)**
No risk factors	Low risk	Moderate risk	High risk
At least one risk factor except diabetes mellitus	Moderate risk	Moderate risk	High risk
TOD/CCD and/or DM	High risk	High risk	High risk

3.2.1 Pathogenesis of hypertension in diabetes mellitus

Several reason are postulated for the development of hypertension in diabetes, one of the reasons that hypertension is very often associated with diabetes is that hyperglycemia accelerates atherosclerosis. We have previously demonstrated that patients with Type 2 diabetes mellitus showed diminished forearm blood flow due to increased vascular resistance even at younger ages **(Ide et al, 1991).** Obesity-related sympathoadrenergic stimulation and insulin resistance might be other reasons for hypertension. **(Kashiwabara, 2000).** Volume expansion derived from diabetic nephropathy is another cause of hypertension. As well as endothelial dysfunction possibly due to hyperglycemia and/or oxidative stress may be also very important to increase blood pressure. Diminished bradykinin and NO synthesis in endothelial cells may also cause hypertension. Although the renin–angiotensin system is not augmented as evidenced by normal to low plasma renin activity, the tissue renin–angiotensin system might be activated in diabetes mellitus which might had to increase in overall BP. (Tooke et al, 1998). Increased urinary albumin excretion is a major prognostic factor both for progressive diabetic renal disease and increased cardiovascular morbidity and mortality rates in both type 1 and type 2 diabetes. Albuminuria in type 2 diabetes is associated with cardiovascular risk factors such as raised blood pressure (BP), dyslipidemia, and endothelial activation. (Mogensen, 2003). Other plausible mechanisms can be postulated: impaired autoregulation and attenuation of the nocturnal decrease of blood pressure. Impaired autoregulation, i.e. the inability of arterioles to contract when blood pressure is increased, leads to increased pressure in the microcirculation, damage to the microvascular endothelium and microvascular sclerosis [24, 25]. This loss of the autoregulatory response can amplify the damaging effects of systemic blood pressure on small blood vessels. (Tooke, 1995)

High blood pressure (BP), obesity, and abnormal lipid profile, which often coexist with diabetes, tend to be associated with preclinical cardiovascular abnormalities and may contribute to the association of diabetes with cardiovascular events. Cardiac features of diabetic and nondiabetic hypertensive subjects remain incompletely

described in population-based samples. Therefore, the compared clinical and metabolic characteristics, LV geometry, and systolic function between diabetic and nondiabetic hypertensive participants in the Hypertension Genetic Epidemiology Network (HyperGEN) Study. (Hilary et al, 1969)

3.3 CARDIOVASCULAR COMPLICATION OF TYPE 2 DIABETICS

Hypertension clearly increases cardiovascular risk in type 2 diabetes, as shown most strikingly by data from the Multiple Risk Factors Intervention Trial. Of the 350 000 men aged between 35 and 57 years who were recruited to this study, the absolute risk of cardiovascular death was three-fold higher in those who were diabetic, even after adjusting for other common risk factors such as age, race, income, serum cholesterol and smoking. Importantly, the risk at any given level of SBP was 2.5–3 times higher in those with type 2 diabetes than in their non-diabetic counterparts at every level of SBP assessed. (Stamler et al, 1993) DIGAMI trial showed that (26%). deaths had occurred in the control group compared to (19%). in the infusion group relative mortality reduction (30%); Among patients without prior insulin treatment and with low cardiovascular risk, mortality was significantly reduced already during the hospital phase, and after 1 year there were (18.0%). deaths in the control group and (8.6%). in the infusion group (relative mortality reduction 52%; These data showed that improvement in diabetes decreases the risk of myocardial infraction.(Malmberg et al, 1996)

Table 3.3.1 Prospective studies showing an association between cardiovascular events and glycemic control in patients with type 2 diabetes (Meigs et al, 1997)

country	Year of publication	Number of patients	Age (years)	Length of follow-up (years)	End point
Finland	1993	133	45 – 64	10	Cardiovascular mortality
Finland	1994	229	65 – 74	3.5	CHD mortality and morbidity
Finland	1994	229	65 – 74	3.5	Stroke incidence
U.S.	1995	1370	> 30	10	CHD mortality and stroke incidence
Denmark	1995	328	20 – 65	5	Cardiovascular mortality

Germany	1996	1139	30 – 55	11	Incidence of myocardial infarction
Germany	1996	290	< 76	10	Cardiovascular mortality
Finland	1996	1059	45 – 64	7	Stroke mortality and morbidity
Finland	1997	1059	45 – 64	7	CHD mortality and morbidity
U.K.	1998	2693	25 – 65	8	CHD mortality and morbidity
U.S.	1998	471	25 – 65	7.5	Cardiovascular mortality

It was found that diabetes-associated increase in LV mass and RWT and decrease in LV mid wall function, which may contribute in part to high rates of overt coronary heart disease and heart failure to which diabetic individuals are predisposed. Although abnormalities of LV geometry and myocardial function were mildly greater in diabetic than nondiabetic hypertensive patients, our results suggest that those differences may amplify over time. This identification of a cluster of abnormalities of LV geometry and function with factors associated with diabetes supports multifactorial approach for preventing high rates of cardiovascular morbidity and mortality in type 2 diabetics. (UKPDS, 1998), (Hansson et al, 1998)

Table 3.3.2 Fasting Laboratory Data in Diabetic and Nondiabetic Hypertensive Subjects (Vittorio et al, 2001)

	Diabetic	Nondiabetic	*P*
Glucose, mg/dL	166±37	98±35	<0.001
Total cholesterol, mg/dL	199±39	202±39	NS
HDL cholesterol, mg/dL	49±15	52±15	<0.001
Triglycerides, mg/dL	157±92	134±91	<0.001
Total cholesterol/HDL cholesterol	4.4±1.4	4.2±1.3	<0.001
Triglyceride/HDL cholesterol	3.92±2.98	2.97±2.50	<0.001
Creatinine, mg/dL	1.07±0.43	0.99±0.28	<0.01

Table 3.3.3 LV Geometry in Diabetic and Nondiabetic Hypertensive Subjects (Vittorio et al, 2001)

	Diabetic	Nondiabetic	*P*
LV mass, g	178±38	173±37	<0.01
LV internal diameter, cm	5.1±0.4	5.1±0.4	NS
Interventricular septum, cm	0.98±0.12	0.95±0.12	<0.005
Posterior wall thickness, cm	0.91±0.12	0.89±0.11	<0.001
RWT	0.36±0.05	0.35±0.05	<0.005

Type 2 diabetes and loss of glycometabolic control is more than just a risk factor for the development of cardiovascular disease. The two pathologies are intimately connected, such that improvements or deterioration in one disease appear to be reflected in the other. Elevated blood glucose, like elevated blood pressure, is on a continuum of risk, therefore it is important to addressing the cardiovascular, thrombotic and hypertensive elements and choose appropriate treatments. (Pedersen et al, 2003)

The most important risk factor for CHD (fatal and nonfatal myocardial infarction, angina pectoris) induced by diabetes 2 was high LDL cholesterol, followed by low HDL cholesterol and HbA1 c, and for nonfatal and fatal myocardial infarction, high LDL cholesterol, followed by diastolic blood pressure, low HDL cholesterol and HbA1 c.

Table 3.3.4 Prevalence of hyperglycemia, elevated blood pressure, and high cholesterol and their risk reduction for different end points (UKPDS 33, 1998)

PATHOLOGY	**RR (Risk Reduction) (%)** **In diabetic patients**
Hyperglycemia Myocardial infraction	100 16
Elevated blood pressure Myocardial infraction Stroke	40 21 44
High cholesterol Coronary heart disease	30-50 30

3.3.1 Pathophysiology of Cardiovascular Complications

Cardiovascular disease (coronary heart disease, myocardial infarction stroke, peripheral vascular disease) is the most important cause of mortality and morbidity among patients with type 2 diabetes. Conventional risk factors contribute similarly to macrovascular complications in patients with type 2 diabetes and nondiabetic subjects, and therefore, other explanations have been sought for enhanced atherothrombosis in type 2 diabetes. In this section the possible pathophysiology of CVS complications in diabetes are discussed. (Markku et al, 1999)

Association of glycometabolic and endothelial dysfunction with vessel disease

The majority of patients with established atherosclerosis have some disturbance of glucose metabolism. Patients with type 2 diabetes have prominent endothelial dysfunction, a recognized first step in the atherogenic pathway. (Cleland et al, 2000) The vascular endothelium is sensitive to insulin, which, in addition to regulating the delivery of nutrients to the vascular bed, has vasodilatory, antithrombotic and growth-inhibiting actions. In individuals with type 2 diabetes, the vascular endothelium is resistant to the actions of insulin. (Ouvina et al, 2001)

These findings implicate insulin resistance in the pathogenesis of vascular disease in patients with type 2 diabetes and suggest that hypoglycaemic therapy in patients with ischaemic heart disease may represent a good target for further research.

Diabetic Cardiovascular Autonomic Neuropathy (DCAN)

DCAN is associated with LV diastolic dysfunction (LVDD) at rest, both in patients with long-term type 2 or type 1 diabetes (Aaron et al, 2007).

The pathophysiology of LVDD includes delayed relaxation, impaired LV filling, and/or increased stiffness (Mandinov et al, 2000). Diabetes mellitus can produce functional, biochemical, and morphological myocardial abnormalities independent of coronary atherosclerosis and hypertension, contributing to heart failure with normal

LV systolic function (Piccini et al, 2004). There may be no evidence of ischemic, hypertensive, or valvular heart disease, yet patients may develop cardiac dysfunction and, finally, congestive heart failure, suggesting the presence of diabetic cardiomyopathy (Sakamoto et al, 1998). In patients with CAN, vagal impairment can lead to a relative predominance of sympathetic activity in the sympathovagal balance. Sympathetic overactivity stimulates the renin–angiotensin–aldosterone system and increases heart rate, stroke volume, and peripheral vascular resistance, thus contributing to LV dysfunction (Perin et al, 2001). The latter is promoted by the deleterious effects of the renin–angiotensin–aldosterone and adrenergic systems on systemic and coronary hemodynamics, myocyte hypertrophy and fibroblast growth, and myocyte necrosis and apoptosis (Chatterjee et al, 2002). Such a sympathetic hyperactivity, in combination with regional myocardial sympathetic denervation, has been shown recently to lead to diminished coronary blood flow reserve and diastolic dysfunction in diabetic patients with early microangiopathy (Pop-Busui et al, 2004). Analogous to the situation with neurohormones, the overexpression of cytokines is sufficient to contribute to LV dysfunction and, eventually, to heart failure (Sekiguchi et al, 2004).

Heart failure

Heart failure (HF) is a common and serious co morbidity of diabetes. Epidemiological

studies carried out over the last 3 decades have established the association between diabetes and HF, the underlying pathophysiological explanation for this common comorbidity is less clear. Several theories characterizing specific cellular or metabolic derangements linking diabetes and HF have been investigated, including a triad of overlapping cardiotoxic and cellular maladaptive alterations comprising a specific diabetes-related cardiomyopathy, association with coronary artery disease, distorted gene expression, and alteration in autonomic activity..

The coexistence of myocardial ischemia, hypertension, and a specific diabetic cardiomyopathy seems to independently and cooperatively contribute to biochemical,

anatomic, and functional alterations in cardiac cells and tissues that impair cardiac function. Results from a series of animal research studies, supported by clinical studies in humans, point to a role for these overlapping influences in patients with diabetes and HF.

The high incidence and poor prognosis of HF in diabetic patients have been linked in part to the presence of an underlying diabetic cardiomyopathy characterized by myocellular hypertrophy and myocardial fibrosis (Factor et al, 1983). Diabetic cardiomyopathy has been found to be associated with depressed mechanical function, electrophysiological abnormalities, defects in subcellular organelles, and receptor downregulation because of chronically elevated catecholamine levels. Experimentally induced diabetes in animal models causes changes in myocardial cellular calcium transport and contractile proteins, which result in subclinical systolic and diastolic dysfunction. The increased myocardial collagen content associated with diabetic cardiomyopathy further worsens diastolic dysfunction (Ganguly et al, 1983).

Hypertension, another frequent comorbidity of diabetes, may further damage myocardial contractile proteins, induce increased myocardial fibrosis, and generate a hypertrophic state, which results in mild clinical systolic and diastolic dysfunction. Furthermore, the addition of myocardial ischemia may change a mildly dysfunctional myocardium, caused by diabetes or a moderately dysfunctional myocardium caused by the combined effects of diabetes and hypertension, to a severely dysfunctional myocardium and even terminal HF. The end result of diabetes, hypertension, and myocardial ischemia is a fibrotic, noncompliant myocardium, initially with diastolic and later with systolic dysfunction. In addition, papillary muscle fibrosis can lead to a mitral insufficiency that adds a mechanical burden to the already dysfunctional myocardium. (Stone et al, 1989)

Although severe myocardial dysfunction in the diabetic patient is often caused by a combination of diabetic cardiomyopathy, hypertension, and/or myocardial ischemia,

any one of these factors may dominate. The appropriate management of diabetic patients with severe HF requires evaluation for coronary artery disease. The absence of significant coronary obstructions in a subset of patients with diabetic HF has suggested the possibility of a diabetic microangiopathy as an underlying etiology, although microvascular ischemia has generally been excluded by the absence of increased lactate production during rapid atrial pacing (Genda et al, 1986). However, it is still possible that in the insulin resistant or diabetic patient, endothelial dysfunction could lead to repeated episodes of vasoconstriction, with subsequent reperfusion injury and myocardial dysfunction. Furthermore, the increased small vessel permeability associated with endothelial dysfunction could lead to interstitial edema, fibrosis, and myocardial dysfunction. It is also possible that a defect in the angiogenic response to ischemia that has been reported in diabetic patients could also play a role (Yarom et al, 1992).

Animals with experimental diabetic cardiomyopathy exhibit biochemical and molecular abnormalities resembling those seen in human myocardial failure stemming from hemodynamic overload, which potentially contribute to HF (Bristow et al, 1998). Hyperglycemia has been shown to activate the same intracellular signaling pathways (e.g, protein kinase C and mitogenactivated protein kinase) as mechanical stretch or increased ventricular wall stress. Regardless of the setting, impaired myocardial performance would eventually require activation of the neurohormonal compensatory systems, including the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS), to avoid systemic hypoperfusion. Activation of these and other autocrine and paracrine systems leads to the progressive loss of cardiac myocytes because of accelerated apoptosis and necrosis, eventuating in further myocardial dysfunction and the downward spiral of cardiac failure. Similarly, activation of the RAS and SNS leads to compensatory changes in the size and shape of the cardiac chambers through cellular hypertrophy, or “remodeling.” Even though this process involves increased cardiac muscle mass, the change in cellular and noncellular composition, geometry, and energetics leads to further decreases of ventricular function and even greater increases in neurohormonal activation. Based on

this proposedscenario, the HF in diabetic cardiomyopathy would appear to follow the same pattern of initially adaptive but eventually harmful compensatory mechanisms leading to progressive ventricular dysfunction, as recognized in HF of other etiologies (Eichhorn et al, 1996).

At a cellular level, activation of the RAS and SNS leads to defects in β-adrenergic receptor signal transduction and induction of the fetal gene program (Dillmann et al, 1986). An important metabolic consequence of β-adrenergic receptor signaling is increased stimulation of carnitine palmityl transferase 1 (CPT-1) activity. CPT-1 is a mitochondrial enzyme that plays a key role in transporting long-chain acyl-CoA compounds into the mitochondria, promoting myocardial fatty acid rather than glucose utilization. Increased myocardial use of free fatty acids (FFAs) results in the uncoupling of oxidative phosphorylation, inhibition of membrane ATPase activity, increased myocardial oxygen consumption, myocardial ischemia, impaired myocardial function, and cardiac arrhythmias. Inhibition of CPT-1 is one mechanism through which β-blockade may be cardioprotective (Panchal et al, 1998).

Another change brought about through β-adrenergic receptor signal transduction abnormalities, and one believed to contribute to HF progression, is an alteration of gene expression to what has been called the fetal gene program. Atrial natriuretic peptide, which is ordinarily limited to atrial muscle, is re-expressed in the ventricle, as it was in fetal life. The proportions of the fast (α) and slow (β) isoforms of myosin heavy chain (MHC) are changed into a more fetal-like pattern with higher β-MHC and lower α-MHC. The skeletal muscle α-actin gene, which is not expressed in cardiac muscle after birth, is also re-expressed in the heart along with the normal cardiac actin gene. As these genes are being re-expressed, there is a downregulation of the gene encoding a key inotropic protein, sarcoplasmic reticular Ca2 ATPase (SERCA-2). The net effect of these changes in gene expression is an overall decrease in both diastolic and systolic ventricular function, which may be an adaptive mechanism to protect the surviving myocardium by reducing its energy expenditure (Bristow et al, 2000).

Altered gene expression is also reversed by β-blockade. Studies in diabetic rats have shown improvement in SERCA-2 expression, as well as other aspects of fetal gene activation, with β-adrenergic inhibition. In humans, β-blockers have been shown to produce a time-dependent improvement in myocardial contractile function by stopping and even reversing the remodeling process. Indeed, the prophylactic use of β-blockers in patients with diabetes, hypertension, or ischemic heart disease has the potential to prevent the initiation of the remodeling process (Lowes et al, 1998).

Diabetic Cardiomyopathy

The cardiomyopathic process associated with diabetes initially manifests as diminished left ventricular compliance in the presence of normal systolic function. Diastolic abnormalities occur in 27% to 69% of asymptomatic diabetic patients in the absence of or with only mild microvascular complications . Coexistent hypertension, which occurs about twice as frequently in diabetic patients as in the general population , results in more severe cardiomyopathy . Hypertension leads to left ventricular hypertrophy and contributes to decreased left ventricular compliance and cause myocardial necrosis cardiomyopathy (Grines at al, 1989).

Association of Atherosclerosis, Endothelial dysfunction of coronary arteries, impaired autoregulatory response of microvessels with type 2 diabetes.

Autopsy studies have shown that diabetic patients have more extensive coronary atherosclerosis. Concomitant hypertension, atherogenic lipoprotein profile, and abdominal obesity contribute to the accelerated atherosclerosis (GUSTO, 1993).

In diabetes, the capacity of the vascular bed to meet myocardial demand may also be impaired by abnormal epicardial vessel tone and microvascular dysfunction.

Dilatation of epicardial arteries in response to hypoxia mainly relies on the release of endothelium-dependent relaxing factor. Impaired endothelium-dependent relaxation found in diabetes and occurs in various vascular beds, including the coronary arteries. Hyperglycemia is the primary mediator of diabetic endothelial dysfunction. Impaired

endothelium-dependent relaxation can be induced by brief exposure (several hours) to high glucose concentrations. Endothelial dysfunction is thought to result primarily from increased generation of free radicals and the presence of advanced glycosylation end products that deactivate nitric oxide (Umans et al, 1995).

The abnormal vasodilatory response associated with diabetes also extends to the microcirculation. Locally regulated microvascular dilatation permits efficient distribution of blood flow in the myocardium . Coronary arterial microvessels dilate in response to graded reductions in coronary perfusion. This autoregulatory response is blunted in hyperglycemic animals and diabetic patients . This functional abnormality may also be related to endothelial dysfunction and can be worsened by structural abnormalities of the coronary microcirculation (Kersten et al, 1995).

Severe and diffuse atherosclerotic disease, endothelial dysfunction of coronary arteries, impaired autoregulatory response of microvessels to increased myocardial demand, and structural changes in the coronary microvasculature can all lead to myocardial ischemia in the surviving myocardium, rendering it unable to compensate efficiently.

Abnormal Myocardial Substrate Metabolism

Decreased GLUT4 translocation caused by insulin deficiency could limit glucose availability, thereby promoting myocardial damage and reducing the compensatory capacity of the noninfarcted myocardium. In addition, insulin deficiency reduces the use of myocardial glucose with a shift toward fatty-acid metabolism. This altered pattern of exogenous substrate use may result in increased consumption of oxygen by the myocardium (Zaninetti et al, 1988).

Specific metabolic abnormalities that are induced by diabetes can adversely affect mechanical performance or increase myocardial vulnerability to ischemic insult and convert it to myocardial infarction.

Diabetes induced thrombolytic resistance and myocardial infarction

Platelets play a key role in reducing the efficacy of thrombolytic therapy and facilitating reocclusion. In the presence of diabetes, platelet aggregation may be accelerated at sites of arterial occlusion. Platelets from diabetic patients have enhanced adhesiveness and hyperaggregability in response to various agonists. In diabetic patients, elevated fractions of activated platelets circulate in the absence of clinically detectable vascular lesions. Enhanced activity of the platelet arachidonic acid pathway with increased synthesis of thromboxane A_2 also occurs in diabetic patients. The increased functional behavior of platelets cause thrombolytic resistance and myocardial infarction (Daniel et al, 1997).

3.4 ANTIHYPERTENSIVE TREATMENT IN DIABETES MELLITUS

Patients with diabetes are at greater risk for developing microvascular and macrovascular complications, particularly coronary artery disease, kidney disease, cardiomyopathy, heart failure, myocardial infarction, and stroke. Individuals with hypertension in diabetes have accelerated progression of these complications, leading to exaggerated cardiovascular morbidity and mortality. Management of hypertension in these patients reduces the risks and progression of diabetes-associated complications (Helmy et al, 2007).

There is unequivocal evidence for a beneficial effect of blood pressure reduction on cardiovascular risk in type 2 diabetes, and these benefits have been demonstrated with all classes of antihypertensive drugs. Following clinical data support this line.

3.4.1 Diuratics

The Systolic Hypertension in the Elderly Program (SHEP) compared blood pressure reduction using chlorthalidone 12.5–25 mg/day with placebo. The risk reduction was associated with, and probably explained by, a greater reduction in SBP in chlorthalidone group (amounting to 9.8 mmHg in the diabetic patients) (Curb et al, 1996). Diuretics causes, an increased permeability of the microvasculature, secondary to a reduction in insulin resistance Permeability is also increased by increasing

endothelin-1 levels, which are stimulated by insulin and an increase in vascular endothelial growth factor. Improvements in cardiac risk factors, especially endothelial dysfunction, diastolic blood pressure, C-reactive protein levels, microalbuminuria, plasminogen activator inhibitor and adhesion molecule levels, increase in LDL and HDL particle size, and decreased vascular smooth muscle cell proliferation is achieved by diuretics therapy should be considered (Fukunaga et al, 2001).

3.4.2 ACE inhibitors

The benefit provided by angiotensin-converting enzyme (ACE) inhibitors is clear. Probably the strongest evidence for the cardiovascular benefit of ACE inhibitors in diabetic patients comes from the diabetic subgroup of the Heart Outcomes Prevention Evaluation trial. Ramipril gave a 22% reduction in the relative risk (RR) of myocardial infarction, a 33% reduction in the RR of stroke, a 37% reduction in the RR of cardiovascular death and a 24% reduction in the RR of overall mortality. There was also a 16% reduction in the RR of microvascular disease (overt nephropathy, laser therapy or dialysis) (HOPE, 2000). ACE inhibition exerts its cardiovascular benefits primarily by blocking the conversion of angiotensin-I to angiotensin-II (ANG-II), thereby decreasing the circulating level and tissue concentration of ANG-II. In addition to being a potent vasoconstrictor, ANG-II induces the protein synthesis involved in cardiac myocyte hypertrophy as well as collagen production by cardiac fibroblasts, leading to myocardial fibrosis (Weber et al, 1991). ACE inhibitors also attenuate cardiac myocyte hypertrophy and myocardial fibrosis by raising bradykinin and prostacyclin levels and mediating the release of nitric oxide. ACE inhibitors can reduce mortality and limit cardiac morbidities, including HF, in diabetic patients with or without systolic dysfunction (Yusuf et al, 2000). One of the mechanisms for improvement is through the prevention of myocardial remodeling. In patients with anterior or inferior wall MIs, increases in left ventricular chamber dimensions and sphericity can be prevented by ACE inhibitors. But cough and angioedema is the main problem with ACE inhibitors.

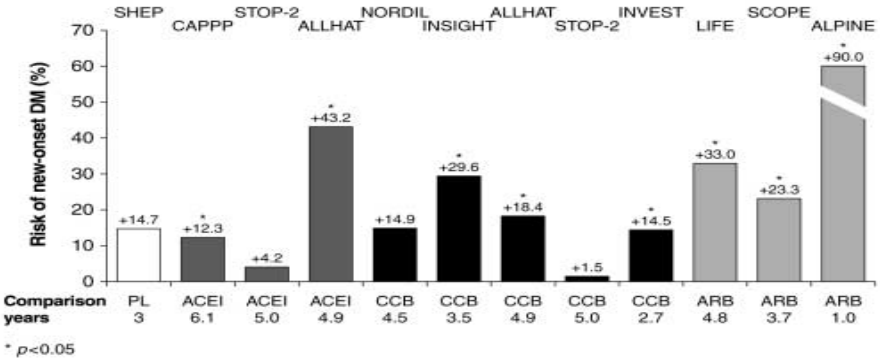
3.4.3 AT-1 Receptor blocker

Evidence from trials, however, also shows a reduced incidence of new-onset diabetes in patients treated with **angiotensin II receptor blockers** as compared with placebo, which suggests a true antidiabetic effect of new drugs . In this regard, some other trials are important. This is the case in particular for the VAlsartan Long-term Use Evaluation (VALUE), which showed a significant benefit for the angiotensin II receptor blocker valsartan. AT-1 receptor antagonist also work through Angiotensin II and have same mechanism but Unlike ACE inhibitors, ANG-II receptor blockers do not increase bradykinin levels and therefore may be less effective in impacting mortality caused by HF.

3.4.4 Calcium channel blockers

Data from the diabetic subgroup in the Systolic Hypertension in Europe trial show that **calcium channel blockers** do provide a protective effect compared with placebo. In the 492 diabetic patients, SBP was reduced by an additional 8.6 mmHg and DBP by an additional 3.9 mmHg in the nitrendipine group compared with the placebo group. This was accompanied by 69% reduction in stroke, a 57% reduction in cardiac events, a 70% reduction in cardiovascular mortality and a 41% reduction in overall mortality in comparison with placebo (Estacio et al, 1998). They hyperpolarized the heart and decreases force of contraction.

Fig. 3.4.1 Incidence of new-onset diabetes mellitus (DM) in large, comparative antihypertensive drug trials comparing newer treatments with conventional one (diuretics and/or beta-blockers) in different clinical study (Curb et al, 1996).



All above antihypertensive classes have same effect throughout all the drugs in that class. But β- blockers have very different therapeutic profile with different drugs in same class.

3.5 β-BLOCKERS AND TYPE 2 DIABETES

Beta-blockers have been convincingly shown to reduce total and cardiovascular morbidity and mortality in hypertensive diabetic patients. After myocardial infarction these agents confer a twice as high protective effect when compared to nondiabetic patients. However, most paradoxically, beta-blocking agents are used less frequently in diabetes. A thorough analysis of the literature does not reveal adverse metabolic effects, a higher risk of hypoglycaemia or less nephroprotective effects of beta1-selective beta-blocking agents, which could justify the reticence in prescribing these antihypertensive agents to diabetic patients. The unnecessary less frequent prescription of beta1-selective beta-blockers in diabetes mellitus contributes to the higher cardiovascular mortality in these patients (Sawicki et al, 2001).

β-blocker treatment in coronary heart disease is less frequently used in diabetic patients. Only 40–50 % of diabetic patients receive β-blockers after myocardial infarction, a considerably lower number than non-diabetic patients (Löwel et al, 2000). Even in a large recently published intervention study in high risk diabetic patients aiming at reduction of cardiovascular mortality, only 28 % received β-blockers. This occurs paradoxically despite the fact that all large intervention studies have convincingly shown that β-blockers in diabetic patients after AMI are more effective than in non-diabetic patients (HOPE, 2000).

Table 3.5.1 Overview of acute (up to 3 months) and long-term (more than 1 year) effects of beta-blocker treatment after acute myocardial infarction (AMI) on relative mortality in patients with and without diabetes (Sawicki et al, 2001).

Study	β-blockers and acute reduction of mortality after AMI (Relative Risk Reduction, %) Non-diabetic patients	Diabetic patients
Göteborg Metoprolol Trial [14]	–36 %	–58 %
MIAMI Trial [15]	–12 %	–50 %
ISIS 1 [16]	–15 %	–22 %
Malmberg et al. [17]	–29 %	–69 %
	β-blockers and long-term reduction of mortality after AMI (Relative Risk Reduction, %)	
BHAT [18]	–25 %	–35 %
Gundersen et al. [19]	–34 %	–63 %
Kjekshus et al. [20]	–49 %	–56 %

Several studies have show that selective β1-blocking agents have adverse effects on glucose metabolism, prolong hypoglycaemia or mask hypoglycaemic symptoms and cause diabetic nephropathy. However Sawicki at el. 2001, reported that there are no clear evidences which gives effect of β- blockers on diabetic induced cardiovascular complications. So the role of beta blockers is unclear in type 2 diabetes.

Heart failure is associated with the harmful effects of chronic SNS activation. Norepinephrine, acting through α1-, downregulated β1-, and mildly upregulated β2-receptors, causes direct myocardial toxicity and stimulates altered gene expression and remodeling (Mann et al, 1992). This is exacerbated in diabetes, wherein insulin resistance and hyperinsulinemia are associated with increased sympathetic tone, as indicated by an elevated heart rate (Festa et al, 2000). Furthermore, high ANG-II levels also increase norepinephrine production, whereas ANG-II itself has a direct toxic effect on cardiomyocytes. To prevent cardiac remodeling most effectively, both neurohormonal systems must be therapeutically blocked. β-Blockade, particularly with nonselective agents, is an effective intervention to inhibit sympathetic activation at both α- and β-receptors and prevent the deleterious effects of norepinephrine on cardiac cells and tissues (Gavras et al, 1975).

3.5.1 Classes of β-blockers for type 2 diabetes

There are three generations of β-blocking agents. The first-generation agents, such as propranolol and timolol, are contraindicated in HF patients because of their myocardial depressant effects. Second-generation β-blockers, including metoprolol

and atenolol, are safe to use in HF, but are selective for β1 activity and therefore of limited efficacy. The third-generation β-blocking agents were developed specifically to act nonselectively to provide more comprehensive benefit, each with a different specificity for β1-, β2-, and α1-receptors.

Selective β_1- blockers (Atenolol and metoprolol)

Atenolol-

Drug Category

- Sympatholytics
- Antihypertensive Agents
- Antiarrhythmic Agents
- Adrenergic Agents

Properties-

- Selective
- Hydrophilic
- With intrinsic sympathomimetic activity (ISA)
- With high membrane stabilizing activity
- Long half-life
- Decreases heart rate, contractility and cardiac output, therefore decreasing blood pressure

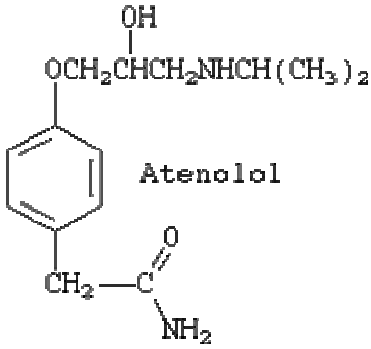
Chemical formula and structure-

$C_{14}H_{22}N_2O_3$

Relative Molecular Mass = 266.3

Chemical name:-

(RS)-4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide



Mechanism of action-

Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension. Higher doses of atenolol also competitively block beta(2)-adrenergic responses in the bronchial and vascular smooth muscles.

Effect on diabetes and cardiovascular complications-

Clinical trial data of SHEP suggested that 19% of diabetic patients received concomitant atenolol, showed beneficial effect of this class of drugs on the diabetes induced hypertension. (SHEP, 2000). Finally, the studies in which atenolol was used for secondary prevention of myocardial infarction show that administration of this drug is protective both in non-diabetic and in diabetic patients (UKPDS 39, 1998). The first International Studies of Infarct Survival trial showed a significant reduction in post-myocardial infarction mortality with atenolol that was comparable in the diabetic and non-diabetic groups (Sleight et al, 1987).

Some experimental study reported that treatment with atenolol in diabetic rats produced insignificant reduction in the elevated triglyceride concentration and coronary events has been reported (Carlson and Botinger 1972, Dharmalingam et al. 2004).

Metoprolol

Drug Category

- Sympatholytics
- Antihypertensive Agents
- Antiarrhythmic Agents
- Adrenergic Agents

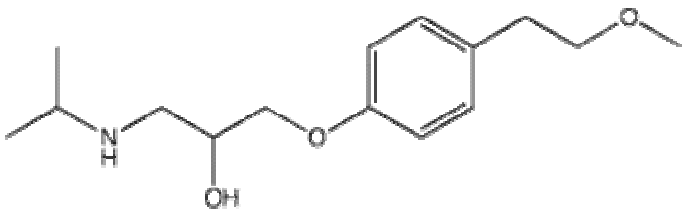
Properties-

- Selective
- Moderately lipophilic
- Without intrinsic sympathomimetic activity (ISA)
- With weak membrane stabilizing activity
- Short half-life, therefore must be taken at least twice daily or as a slow-release preparation
- Decreases heart rate, contractility and cardiac output, therefore decreasing blood pressure

Chemical formula and structure-

1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol

$C_{15}H_{25}NO_3$



Mechanism of Action

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle. Beta(1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Effect on diabetes and cardiovascular complications-

In the diabetic subgroup of two large trials of metoprolol in post-myocardial infarction, the Göteborg Metoprolol Trial (GMT) and the Metoprolol In Acute Myocardial Infarction (MIAMI) study, the reduction in mortality and late infarction

was even greater than in the non-diabetic groups, with a 58% reduction of diabetic mortality in GMT and a 50% reduction in MIAMI (Malmberg et al, 1989). Various clinical trials carried out with metoprolol, such as the BCAPS, SLVA, GMT, MIAMI, MAPHY and SMT trials, suggest the positive effects of metoprolol in various cardiovascular diseases (DeSouza, 2009).

But some clinical and experimental data shows that therapeutic and adverse effect profile of atenolol and metoprolol is indifferent in DM induced cardiovascular complications (Bristow et al, 2000).

In experimental study nonselective β-blokers have been shown to adversely affect serum lipid levels. Although this is less likely to occur with cardioselective β_1-blokers such as atenolol and metoprolol, atenolol nonetheless failed to improve cardiac performance or reduce lipid levels in diabetic patients. Moreover, it caused depression of cardiac function, worsened cardiomyopathy, and caused a number of degenerative changes in the left ventricular tissue of the diabetic heart.

The lipophilic agents metoprolol, timolol, and propranolol have been shown to decrease mortality in coronary artery diseases, particularly from sudden cardiac death. Metoprolol is a relatively lipophilic β_1-bloker devoid of any intrinsic sympathomimetic activity and having only weak membrane-stabilizing activity. Metoprolol produced antihypertensive, antiarrhythmic, antianginal, antiatherosclerotic and cardioprotective activeties. It does not produce any significant adverse effects on insulin secretion, glucose tolerance, or lipids and lipoprotein metabolism in hyperinsulinemic subjects with essential hypertension (DeSouza, 2009).

Despite many reports on the beneficial effects of metoprolol over atenolol in cardiovascular diseases, there are only a few studies carried out in diabetic animals.

β1, β2 and α1 selective β- blockers

Carvedilol-

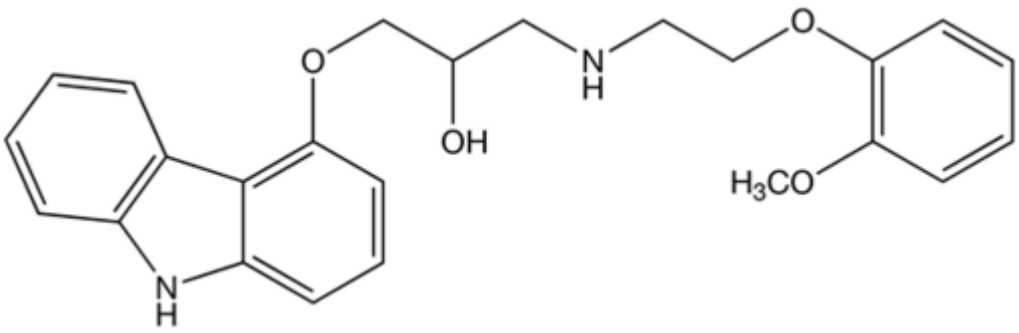
Drug category-

- Sympatholytics
- Antihypertensive Agents
- Antiarrhythmic Agents
- Adrenergic Agents

Properties-

- β-1 and α-1 selective blocker
- Minimal intrinsic activity

Chemical formula and structure-



1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol

$C_{24}H_{26}N_2O_4$

Mechanism of action-

carvedilol results largely from α_1-adrenoceptor blockade, and its β-adrenoceptor blocking activity prevents reflex tachycardia. In some regional vascular beds, such as the cutaneous circulation, the calcium-channel blocking activity of carvedilol may be responsible for increasing the blood flow.

Effect on diabetes and cardiovascular complications-

AVID and COMET trials suggested that carvedilol shown a good results in LV remodeling in CHF patients (Mitchell et al. 2003 and Poole-Wilson et al. 2003).

The GEMINI trial will assess whether differences in glycemic control are seen when the second-generation β-blocker metoprolol, or the third-generation h-blocker carvedilol, is utilized in patients with hypertension and Type 2 diabetes. Metoprolol is expected to increase insulin resistance and worsen glycemic control, whereas carvedilol is expected to be neutral or slightly improved glycemic control. Glycemic control, assessed by the change from baseline in HbA1c after 5 months of maintenance treatment, should be independent of differences in blood pressure lowering. Carvedilol is also expected to improve other parameters of insulin resistance, with the reverse occurring with metoprolol. Fasting plasma glucose, serum insulin, and insulin resistance assessed by the HOMA method will be compared, as will changes in triglycerides, HDL, LDL, and urinary ACR (George et al, 2005).

The GEMINI trial will also compare the side-effect profile of these second- and third-generation h-blockers. Fatigue, cold extremities, and sexual dysfunction have been reported to be less with third-generation h-blockers. Because peripheral vascular disease and erectile impotence due to autonomic neuropathy are more common in the diabetic patient, the potential for their reduced frequency with carvedilol is of great importance (Bell et al, 2003).

The above findings regarding second generation beta 1 antagonists and third generation beta antagonists are still insufficient to establish their complete profile of cardiovascular complications. Hance the objective of the present study is to compare and evaluate the effects of carvedilol, metoprolol and atenolol on cardiovascular complications associated with streptozotocin induced neonatal model of type II diabetes mellitus in rats.

4. MATERIAL AND METHODS

4.1 PROTOCOL

The protocol of the experiment have been approved by institutional animal ethics committee as per the guidance of committee for the purpose of control and Supervision of experiments on animals (CPCSEA), Ministry of social justice and Empowerment, Government of India. (IPS/PCOL/MPH10/003, 23 January 2010)

4.2 MATERIALS

All chemicals and drugs: sodium citrate, STZ, p-dimethyl amino benzaldehyde, glucose diagnostic diagnostic kit, LDL measurement diagnostic diagnostic kit, triglyceride diagnostic diagnostic kit, cholesterol diagnostic diagnostic kit, insulin diagnostic diagnostic kit, saline, C-Reactive protein, CKMB, LDH diagnostic diagnostic kit. All diagnostic diagnostic kits were purchased by Labcare diagnostic Pvt Ltd., India.

4.3 INDUCTION OF TYPE- 2 DIABETES MELLITUS:

Wistar rats from an inbred colony were bred under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and 12h/12h light-dark cycle. Conventional laboratory diet and tap water were provided *ad libitum*.

Five-day-old neonates of either sex were injected intraperitoneally (i.p.) with 85 mg/kg STZ (Sigma Ltd., USA) in 0.9% sodium chloride solution. Control neonates received equivalent amount of isotonic saline alone. The neonates were left with their own mothers and weaned at four weeks of age. Twelve weeks after the injection of STZ, animals were checked for fasting glucose levels with the help of available diagnostic diagnostic kit (Labcare Diagnostics Pvt. Ltd., India). The animals showing fasting glucose levels >170 mg/dl were considered as diabetic.

4.4 TREATMENT PROTOCOL

The rats were then randomly divided into eight groups as follows:

CON - Control rats

COC - Control rats treated with carvedilol

COM - Control rats treated with metoprolol

COA - Control rats treated with atenolol

DIB - Diabetic control rats

DIC - Diabetic rats treated with carvedilol

DIM - Diabetic rats treated with metoprolol

DIA - Diabetic rats treated with atenolol

Carvedilol, metoprolol and atenolol were dissolved in distilled water and were administered orally (p.o.) at a dose of 1mg/kg/day, 10 mg/kg/day and 10 mg/kg/day respectively for 8 weeks. Animals were maintained for 8 weeks treatment period with free access to conventional dietary feed and water ad libitum. All animals were monitored regularly for changes in body weight, food intake, water intake and mortality throughout the course of study.

4.5 BLOOD SAMPLE COLLECTION AND SERUM ANALYSIS:

Blood samples were collected in clean dry centrifuge tubes as the end of 8 weeks of treatment in type 2 diabetic rats after 12h fasting from the retro orbital plexuses under light ether anesthesia and were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 5000rpm for 20 min and stored at -20^0C until the analysis was carried out. Serum samples were analyzed for glucose, cholesterol, HDL- cholesterol, triglycerides, C-Reactive Protein (CRP), Lactate De-Hydrogenase (LDH), Creatinine Kinase (CK) spectrophotometrically (Shimadzu UV-1601, Japan) using available biochemical diagnostic diagnostic kits (Labcare Diagnostics Pvt. Ltd., India). Serum insulin was estimated by radioimmunoassay technique using diagnostic kits obtained from Board of Radiation and Isotope Technology, Mumbai, India in

gamma counter (Packard, USA). Hemodynamic parameter viz. blood pressure, heart rate, rate of pressure development and decay were recorded by carotid artery cannulation using (Labscrib System Inc., USA). After withdrawal of blood samples from retro-orbital plexus and recording hemodynamic parameters, animals were sacrificed, hearts were excised, extraneous tissues were separated and wet weight of the entire heart and left ventricle was noted down to calculate the index of cardiac hypertrophy as wet heart weight to body weight ratio and left ventricular hypertrophy index as left ventricular weight to heart weight ratio.

4.6 ESTIMATION OF VARIOUS SERUM BIOCHEMICAL PARAMETERS:

Serum biochemical parameters:

Glucose, insulin, Lipid profile (cholesterol, Very low density lipoproteins, atherogenic index, triglycerides and High density lipids), Cardiac biomarkers (C-Reactive Protein (CRP), Lactate De-Hydrogenase (LDH), Creatinine Kinase (CK)).

Hemodynamic parameters: Blood pressure and heart rate will be recorded.

Cardiovascular complications: cardiac hypertrophy index and left ventricular hypertrophy index were calculated and Antioxidant and prooxidants was determined from left ventricle (LV).

Histopathological parameters: Slides of heart tissue will be examined for cardiac damage

4.6.1. Serum Biochemical Estimations

Collection of serum:

The blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. It was centrifuged at 2500 rpm for 20 minutes. The serum obtained was kept at 4ºC until used.

4.6.1.1 Estimation of glucose

In vitro quantitative measurement of Glucose concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoeimine dye as indicator. Glucose is a

major source of energy for most cells of the body; insulin facilitates glucose entry into the cells. Diabetes is a disease manifested by hyperglycemia; patients with diabetes demonstrate an inability to produce insulin.

Procedure-

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (Glucose) as shown below:

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix & Incubate for 15 min. at 37°C or 30 min. at R.T. Measure absorbance of Sample (AT) and Standard (AS) against Reagent Blank at 505 nm. The colour is stable for 30 min. at R.T.

Total Glucose (mg/dl) = AT/AS x conc. Standard
Concentration of Std. = 100 mg/dl

4.6.1.2 Insulin estimation:

Insulin was estimated by radioimmunoassay technique using diagnostic kits obtained from Board of Radiation and Isotope Technology, Mumbai, India in gamma counter (Packard, USA).

Principle:

The radioimmunoassay method is based upon the competition of the unlabelled insulin in the standard or samples and radio iodinated (I-125) insulin for the limited binding sites ona specific antibody.

At the end of incubation, the antibody bound and free insulin are separated by the second antibody-PEG aided separation method. Insulin concentration of samples is quantitated by measuring the radioactivity associated with the bound fraction of sample and standard.

Procedure

Tube	Assay buffer	Insulin std.	Serum sample	Insulin free sample	Insulin antiserum	INCUBATE ALL OVERNIGHT 2^0c	I-125 insulin	INCUBATE ALL ALL AT ROOM T^0 AT 3 h	Secondary antibody	PEG	INCUBATE 30 MIN AND CENTRIFUGE 1500 gm
Total	-	-	-	-	-		0.1		-	-	
Blank	0.4			0.1			0.1		0.1	0.1	
Zero std.	0.3			0.1	0.1		0.1		0.1	0.1	
7.5µU/ml	0.2	0.1F		0.1	0.1		0.1		0.1	0.1	
12.5µU/ml	0.2	0.1E		0.1	0.1		0.1		0.1	0.1	
25µU/ml	0.2	0.1D		0.1	0.1		0.1		0.1	0.1	
50µU/ml	0.2	0.1C		0.1	0.1		0.1		0.1	0.1	
100µU/ml	0.2	0.1B		0.1	0.1		0.1		0.1	0.1	
200µU/ml	0.2	0.1A		0.1	0.1		0.1		0.1	0.1	
`sample	0.3		0.1		0.1		0.1		0.1	0.1	

Calculation

y = -0.116x + 33.42

y = B/T %

x = con of insulin (µU/ml)

4.6.1.3. LIPID PROFILE

- **Estimation of total cholesterol:**

In vitro quantitative determination of the activity of cholesterol in serum was done using enzymatic diagnostic kit **(Lab Care Diagnostics, India Limited).**

Principle

Cholesterol esters are hydrolyzed by Cholesterol esterase to produce cholesterol. Hydrogen Peroxide is then produced from oxidation of cholesterol by cholesterol oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4 - aminoantipyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the sample.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Total Cholesterol (TC) as shown below:

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix, Incubate 5 mins at 37°C (or 10 mins at 20 - 25°C) Measure absorbance of the sample (AT) and standard (AS) against reagent blank at 505 nm. The colour is stable for 90 mins at 20 - 25°C

Calculations:

Total Cholesterol (mg/dl) = Abs. of TC / Abs. of Std. X 200 mg/dl

- **Estimation of triglycerides:**

In vitro quantitative measurement of triglyceride (neutral fat) concentration in serum was done by using diagnostic kit **(Lab Care Diagnostics, India Limited)**.

Principle:

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinonemine indicator is formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Triglycerides (TG) as shown below;

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix, Incubate 5 mins at 37°C (or 10 mins at 20 - 25°C) Measure absorbance of the sample (AT) and standard (AS) against reagent blank at 505 nm. The colour is stable for 30 mins at 20 - 25°C

Calculations:

Triglycerides (mg/dl) = Abs. of TG / Abs. of Std. X 200 mg/dl

- **Estimation of LDL Cholesterol:**

In vitro quantitative measurement of LDL-C concentration in serum was done by using diagnostic kit **(Lab Care Diagnostics, India Limited)**.

Principle:

Direct determination of serum LDL-C (low density lipoprotein cholesterol) levels without the need for any pre-treatment of centrifugation steps. The assay takes place in two steps.

-1° elimination of lipoprotein non-LDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

-2° measurement of LDL-C

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

$$2\ H_2O_2 + \text{TOOS} + \text{4-AA} \xrightarrow{\text{POD}} 2H_2O + O_2$$

The intensity of color formed is proportional to the LDL-C concentration in the sample.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Triglycerides (TG) as shown below:

	Blank	Standard	Sample
R1 (µl)	300	300	300
Standard (µl)	-	4	-
Sample (µl)	-	-	4
Mix and incubate for 5 mins at 37°C			
R2 (µl)	100	100	100

Mix and incubate for 5 min at 37^{o}C and read the absorbance against blank at wavelength 546 nm.

Calculations:

LDL-C (mg/dl) = Abs. of sample / Abs. of Calibrator X Calibrator conc.

- **Estimation of HDL Cholesterol:**

In vitro quantitative measurement of HDL-C concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle:

Direct determination of serum HDL-C (High Density Lipoprotein Cholesterol) levels without the need for any pre-treatment or centrifugation of the sample. The method depends on the properties of a detergent which solubilizes only the HDL so that HDL-C is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoprotein LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to abruption of the detergents on

their surfaces. The intensity of the color formed is proportional to the HDL-C concentration in the sample.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Triglycerides (TG) as shown below:

	Blank	Standard	Sample
R1 (µl)	300	300	300
Standard (µl)	-	3	-
Sample (µl)	-	-	3
Mix and incubate for 5 mins at 37°C. Read absorbance A1.			
R2 (µl)	100	100	100

Mix and incubate for 5 min at 37^{o}C and read the absorbance A2 against blank at wavelength 600 nm. Calculate the increase of the absorbance ΔA=A2-A1.

Calculations:

HDL-C (mg/dl) = ΔA sample / ΔA Calibrator X Calibrator conc.

Estimation of VLDL Cholesterol: (Russell et al., 1990)

Estimation of VLDL-cholesterol was done using the Friedwald's formula.

VLDL cholesterol = triglycerides / 5.

4.6.1.4 CARDIAC BIOMARKERS:

- **Estimation of Lactate dehydrogenase:**

In vitro quantitative measurement of LDH concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

Lactate is oxidised to Pyruvate in the presence of NAD by the action of lactate dehydrogenase. The rate of formation of NADH is directly proportional to LDH concentration.

Procedure

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (LDH) as shown below:

Working reagent (Rw)- Mix 9 ml of Buffer reagent with 1 ml of Enzyme reagent.

	Blank	Standard	Sample
Rw (µl)	1000	1000	1000
Standard (µl)	-	25	-
Sample (µl)	-	-	25

Mix well and after 1 min incubation, measure the change in absorbance per min. (Δ A/min.) for next 2 minutes at 340 nm.

Calculations:

Activity (U/L) = Δ A/min x 6592

- **Estimation of creatinine kinase:**

In vitro quantitative measurement of CKMB concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

This procedure involves measurement of Creatine kinase (CK) activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of CKMB while not affecting the B subunit of CK-MB and CK-BB. Than the CK method is used to quantitatively determine CK-BB activity. CK catalyses the reaction between creatine phosphate and ADP, giving creatine and ATP. ATP and glucose in the presence of G6PDH oxidises, and reduces NAD to NADH. The rate of NADH formation is determined photometrically at 340 nm & is directly proportional to CK-BB activity. The CK-MB activity is calculated by multiplying CKBB x 2.

Clinical significance-

CK-MB is an enzyme formed by the association of two subunits from muscle (M) and nerve cells (B). CK-MB is usually present in serum at low concentration; it is increases after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage.

Procedure

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (CKMB) as shown below:

Working reagent (Rw)- Mix 4 ml of Enzyme Reagent I with 1 ml of Enzyme Reagent II.

	Blank	Standard	Sample
Rw (µl)	1000	1000	1000
Standard (µl)	-	50	-
Sample (µl)	-	-	50

Mix well and after 10 min. at 37°C. Measure the change inabsorbance. Repeat readings every minute for next 5 minutes. Calculate Δ A/min at 340 nm.

Calculation-

Δ A/min. x 3376 = U/l CKBB

CKMB = CKBB x 2

- **Estimation of CRP-Turbilatex:**

In vitro quantitative measurement of CRP-Turbilatex concentration in serum was done by using diagnostic kit (Lab Care Diagnostics, India Limited.).

Principle-

The CRP-Turbilatex is a quantitative turbidimetric test for the measurement of C-reactive protein (CRP) in human serum or plasma. Latex particles coated with specific anti-human CRP are agglutinated when mixed with samples containing CRP. The agglutination cause an absorbance change, dependent upon the CRP content of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration. CRP is an acute-phase protein present in normal serum, which increases significantly after most forms of tissue, bacterial and virus infections, inflammation and malignant neoplasia. During tissue necrosis and inflammation resulting from microbial infections, the CRP

concentration can rise up to 300 mg/L in 12-24 hours.

Procedure-

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (CRP) as shown below:

Working reagent (Rw)- Mix 1 ml Latex Reagent + 9 ml Diluent

	Blank	Standard	Sample
Rw (µl)	1000	1000	1000
Standard (µl)	-	5	-
Sample (µl)	-	-	5

Adjust the instrument to zero with distilled water. Mix and read the absorbance at 540 nm after 10 Seconds (A1) and after 2 minutes (A2) of the sample addition.

4.6.2 Cardiovascular Parameters-

- **Physical parameters**

After measurement of hemodynamic parameters, animals were sacrificed. The skin was quickly incised at the midline over the sternum and the heart was exposed by cutting the pericardium. Heart was isolated from the body, blotted with filter paper to remove excess of water, remaining extraneous tissues were removed and weight of the heart was noted down to calculate the index of cardiac hypertrophy as wet heart weight to femur length ratio and weight of the left ventricles was noted down to calculate the index of left ventricular hypertrophy as wet left ventricle weight to wet heart weight ratio. Some other weight ratio like heart weight to body weight and LV to RV weight ratio were estimated. Wall thickness of left ventricular wall was also calculated.

Measurement of cardiac hypertrophy

1. Cardiac hypertrophy index = heart weight/femur length
2. Left ventricular hypertrophy index = LVW/HW
3. Wall thickness in (mm)
4. RVW/LVW ratio

- **Parameters for measurement of cardiac oxidative stress-**

Heart, kept in cold conditions (precooled in inverted petridish on ice) was removed. It was cross chopped with surgical scalpel into fine slices and was chilled in the cold 0.25 M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10%w/v) with 25 strokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for other enzymes assays.

- **Superoxide dismutase:**

SOD was estimated by the method of **Mishra and Fridovich, 1972**.

Principle:

Rate of auto oxidation of epinephrine & the sensitivity of this auto oxidation to inhibition by SOD were augmented as pH was raised from 7.8 – 10.2, O_2, generated by xanthine oxidase reaction, caused by the oxidation of epinephrine to adrenochrome & the yield of adrenochrome produced per O_2 introduced. The auto oxidation of epinephrine proceeds by least two distinct pathways only one of which is free radical chain reaction involving O_2 & hence inhabitable by SOD.

Reagent:

1. Carbonate buffer (0.05 M pH 10.2):
   16.8 gm of $NaCO_3$ was dissolved in 500 ml of distill water & the final volume was made up to 1000 ml with distill water.
2. EDTA 0.49 M:
   1.82 gm of EDTA was dissolved in 1000 ml of distill water.
3. Epinephrine (3 mM):
   0.99 gm of epinephrine bitartarate was dissolved in 1000 ml of distill water.
4. SOD standard:
   Dissolve 1 mg (1000 units /mg) of SOD from bovine liver in 100 ml of carbonate buffer.

Procedure:

Before starting the estimation, see all the reagents required must be kept in freeze and add in cold condition.

Blank	**Test**
5 ml of	5 ml of tissue in 6N HCl
1 ml of TCA (10%)	1 ml of TCA (10%)

Cool for 10 min and centrifuged at 2000 rpm take 0.5 ml of supernatant	
0.5 ml of ST	0.5 ml of ST
4 ml DTNB	4 ml DTNB
1.5 ml Phosphate buffer	1.5 ml Phosphate buffer

The reaction was initiated by the addition of epinephrine and the change in optical density / min was measured at 480 nm, take for 3 min with 30 second interval.

Calculation:

$$SOD = (0.025 - \mathbf{Y}) \div (Y \times 50) \times 100$$

Y = Final reading – Initial reading

Units = Units/ mg of protein.

- **Reduced glutathione:**

Reduced of glutathione (GSH) was estimated by the method of **Moran et. al., 1979.**

Principle:

Glutathione present in RBC consist of sulfhydryl groups. 5,5 dithiobis 2- nitro benzoic acid (DTNB), A disulphide compound, gets readily attacked by these sulfhydryl groups and forms a yellow coloured anion which measured colorimerically at 412 nm.

Reagents:

1. Trichloroacetic acid (10%):
Dissolve 10 gm of TCA in 100 ml of distill water.

2. Dithiobis nitro benzoic acid (DTNB) :
Dissolve 40 gm of DTNB in1% Sodium citrate solution.

3. Phosphate buffer (0.2 M, pH 8.0) :

Dissolve 1.36 gm of KH_2PO_4 in 100 ml of distill water and dissolve in 0.8 gm NaOH in 100 ml distill water.

4. Reduced of glutathione standard :

Dissolve 10 gm of GSH standard in 100 ml of distill water (100µg/ml).

Procedure:

Blank	**Test**
1 ml of D.W.	1 ml of Homogenate
1 ml of TCA (10%)	1 ml of TCA (10%)
Cool for 10 min and centrifuged at 2000 rpm take 0.5 ml of supernatant	
0.5 ml of ST	0.5 ml of ST
2 ml sodium hydrogen phosphate	2 ml sodium hydrogen phosphate
0.25 ml DTNB	0.25 ml DTNB

Mix well keep for at RT read the absorbance against blank at 412 nm using spectrophotometer.

Calculation: **Y = 0.0002X + 0.0049**

X = Conc. of reduced of glutathione

Y = Abs of test sample.

Units: µg of GSH / mg of protein.

- **Lipid peroxidation:**

Malondialdehyde formation (MDA) was estimated by the method of **Ohkawa et al., 1979.**

Principle:

The method estimates Malondialdehyde (MDA), a product of lipid per oxidation process. One molecule of MDA reacts with two molecules of thiobarbituric acid

(TBA) under mildly acidic conditions to form a pink coloured chromogen, whose intensity was measured colorimetrically at 535 nm.

Reagents:

1. Thiobarbituric acid (1% in Tris hydrochloride, pH 7):
   1 gm of thiobarbituric acid was dissolved in 100 ml of Tris hydrochloride buffer pH 7.
2. Trichloroacetic acid (10%):
   10 gm of trichloroacetic acid was dissolved in distilled water.
3. SLS (8%)
   8 gm of SLS in 100 ml of water.

Procedure:

Blank	**Test**
0.2 ml of D.W.	0.2 ml of Homogenate
0.2 ml of SDS	0.2 ml of SDS
1.5 ml acetic acid in HCl	1.5 ml acetic acid in HCl
1.5 ml TBA	1.5 ml TBA
0.6 ml DW	0.6 ml DW
Heated for 45 min in water bath at 95^0 C and cool	
2ml mixture + 2 ml TCA	2ml mixture + 2 ml TCA
Centrifuge on 1000 rpm for 5 min	
Pink color measure at 532 nm	

A = a * b * c

A = abs.

a = mol. Extinction coefficient ($1.56 * 10^5$ cm^{-1})

b = Path length (1 cm^2)

c = con of sample

Units: nm of MDA / gm of tissue.

4.6.3 Hemodynamic parameters-

The animals were anaesthetized by Ketamine (100 mg/kg, i.p.) + Xylazine (7 mg/kg, i.m.). The body temperature was maintained at 37 ± 1 °C during the experiment. The carotid artery behind the trachea was exposed and cannulated for the measurement of hemodynamic parameters using a transducer (BP 100) and Labscrib Systems (I-woks, New Hampshire, USA). The hemodynamic parameters observed were systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP), rate of pressure development (dp/dt_{max}), rate of pressure decay (dp/dt_{min}) and heart rate. All the data were analyzed using labscrib software (Version 118)

4.7 STASTICAL ANALYSIS:

All the values are expressed as mean ± S.E.M. Statistics was applied using SPSS software version 16.0. Statistical significance between normal control and induced control groups was tested using student's t-test. One way ANOVA followed by Dunnett's test was used to determine the statistical significance between induced and test groups. Differences were considered to be statistically significant when $P< 0.05$.

4.8. FIXATION AND PROCESSING OF TISSUES FOR HISTOLOGICAL STUDIES

Liver and aorta were collected after the rats were sacrificed. After blotting free of blood and tissue fluids, were fixed in 10% formalin solution. After 24 hours the tissues were washed thouroughly in repeated changes of 70% alcohol and then dehydrated in ascending grades of alcohol (70-100%). Dehydration in absolute alcohol was followed by treatment of tissues with toluene: xylene (50:50) followed by 10%, 50%, 70%, 90% paraffin wax in toluene and finally 2 changes in 100% wax (paraffin wax, 60-62ºC) followed by embedding of tissue in wax.

5-15µm thick section were serially cut on a leitz microtome in horizontal plane and mounted in glass slide with the help of egg albumin in glycerine solution (50% v/v). The sections were deparafinned in xylene and down graded through 100%, 90%, 70%, 50%, & 30% alcohol and finally in water. They were then stained with 10%

hematoxylin for 3-5 minutes and the staining was intensified by placing in running water. The hematoxylin stained sections were stained with 10% eosin for 2 minutes and were then quickly passed through ascending grades of alcohol and finally treated with xylene followed by mounting in DPX.

The sections were observed and desired areas were photographed in an Olympus photomicroscope for morphometric studies of different cells. The sections were viewed under 40X and 100X magnification and cell measurements were taken with a calibrated ocular micrometer.

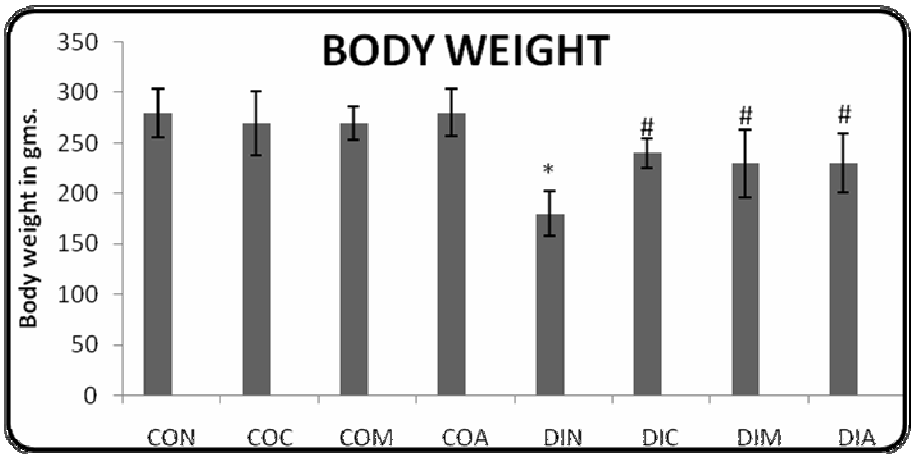
The biopsy study of all the heart of all groups was carried out in accutest research laboratory (india) pvt. Ltd.

5. RESULTS

5. STZ Induced neonatal model of type 2 Diabetes Mellitus

5.1 Effects on general features (Body Weight, Food Intake and water intake)

Diabetic rats showed significant decrease (P<0.05) in body weight at the end of 8th week as compared to control group. Administration of carvedilol, metoprolol and atenolol showed a significant increase in body weight. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter thebody weight of control rats. (Table 5.1, Figure 5.1.A) Diabetic rats also showed significant increase (P<0.05) in food intake and water intake at the end of 8th week as as compared to control group. Administration of carvedilol, metoprolol and atenolol showed a significant decrease in food intake and water intake. Neither of the drug produced any change in food and water intake of control rats. (Table 5.1, Figure 5.1.B)



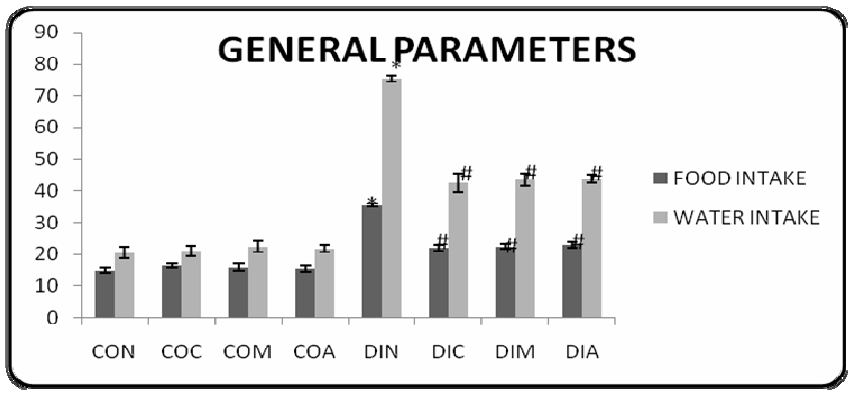


Figure 5.1.A Effects of carvedilol, metoprolol and atenolol on Body weight.

Figure 5.1.B Effects of carvedilol, metoprolol and atenolol on food intake and water intake.

Each Bar contains 6 numbers of animals
Values are expressed as Mean ± SEM

* indicates significantly different from control (P<0.05)

# indicates significantly different from diabetic control group (P<0.05)

CON- control animals, COC- control treated with carvedilol, COM- control treated with metoprolol, COA- control treated with atenolol

DIB- Diabetic control animals, DIC- Diabetic treated with carvedilol, DIM- Diabetic treated with metoprolol, DIA- Diabetic treated with atenolol

5.2 SERUM BIOCHEMICAL PARAMETERS

5.2.1 Effect on glucose and insulin

Streptozotocin-diabetic rats were found to exhibit significant (P<0.05) hyperglycemia as compared to control rats. Chronic treatment with metoprolol and carvedilol produced significant (P<0.05) effect on elevated serum glucose levels. Atenolol significantly (P<0.05) change glucose level in diabetic rats compared to control rats. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the glucose levels of control rats. (Table 5.2.1, 5.2.1.A)

Streptozotocin-diabetic rats were found to exhibit significant (P<0.05) hyperinsulinemia as compared to control rats. Treatment with metoprolol and carvedilol produced significant (P<0.05) effect on elevated serum insulin levels. Atenolol significantly (P<0.05) changes insulin levels in diabetic rats compared to control rats. Neither of the drug produced any change in insulin level of control rats. (Figure 5.2.1.B).

5.2.2 Lipid profile

Effects on serum Cholesterol, VLDL, atherogenic index, Triglyceride and HDL-C

There was a significant ($P<0.05$) increase in cholesterol, very low density lipoprotein (VLDL), atherogenic index and triglycerides levels, and significant ($P<0.05$) decrease in high density lipoprotein (HDL)-cholesterol levels in STZ diabetic rats as compared to control rats.

Administration of carvedilol and metoprolol showed a significant ($P<0.01$) dose-dependent reduction in levels of cholesterol, VLDL-C and atherogenic index and TC, as compared to diabetic group (Figure - 2). Also, a remarkable increase in HDL-C levels was seen with the chronically treated animals. But there is significant ($P<0.05$) changes in cholesterol, VLDL-C and atherogenic index and TC as compared to control group. And changed level of HDL-C was found in atenolol treated group. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the cholesterol, very low density lipoprotein (VLDL), atherogenic index and triglycerides levels and high density lipoprotein (HDL)-cholesterol levels of control rats.

5.2.3 Serum cardiac biomarkers

Effect of serum creatinine kinase, Lactate dehydrogenase and C-Reactive protein

Streptozotocin-diabetes produced a significant ($P<0.05$) increase in serum LDH and CK levels as compared to control rats. Chronic treatment with atenolol, metoprolol and carvedilol significantly ($P<0.05$) changes the elevated serum LDH levels and CK levels of diabetic rats. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the LDH and CK Levels of control rats. (Table 5.2.3, Figure 5.2.3.B & C).

Streptozotocin-diabetes also produced a significant ($P<0.05$) increase in serum CRP level as compared to control rats. Chronic treatment with atenolol, metoprolol and carvedilol significantly ($P<0.05$) alter the elevated CRP levels of diabetic rats. Neither of the drug produced any change in CRP levelsof control rats. (Table 5.2.3, Figure 5.2.3.A)

5.3 CARDIOVASCULAR PARAMETERS

5.3.1 Effect on cardiac hypertrophy index, left ventricular hypertrophy index, LVW/RVW, wall thickness and LVW/TBW ratio.

Diabetic rats exhibited reduced wet heart weight as compared to nondiabetic rats. However, the ratio of heart weight to femur length which is a measure of cardiac hypertrophy was significantly ($P<0.05$) higher diabetics as compared to those of control rats. Further, left ventricular weight-to-heart weight ratio which is a measure of left ventricular hypertrophy index was also significantly ($P<0.05$) high in diabetic control animals as compared to nondiabetic control animals. Chronic treatment with atenolol, metoprolol and carvedilol significantly ($P<0.05$) changes the elevated cardiac hypertrophy index and left ventricular hypertrophy index of diabetic rats. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the of control rats. Cardiac hypertrophy index and left ventricular hypertrophy index (Table 5.3.1, Figure 5.3.1.A & B).

Wall thickness, LVW/TBW and LWV/RVW ratios were also found significantly higher in diabetic rats as compared to nondiabetic control rats. Chronic treatment with atenolol, metoprolol and carvedilol significantly ($P<0.05$) alter the elevated Wall thickness, LVW/TBW and LWV/RVW ratios of diabetic rats. Neither of the drug produced any change in Wall thickness, LVW/TBW and LWV/RVW ratios of control rats.

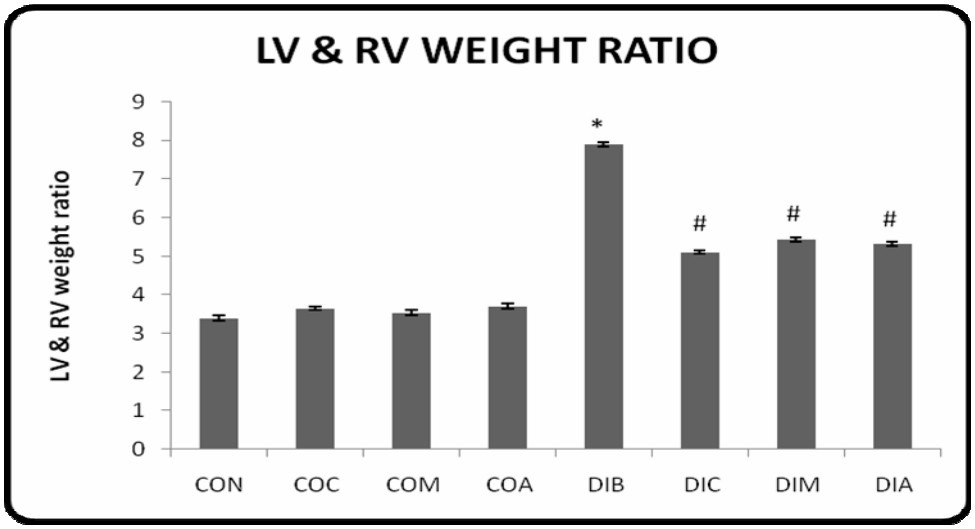


Figure 5.3.1.D.

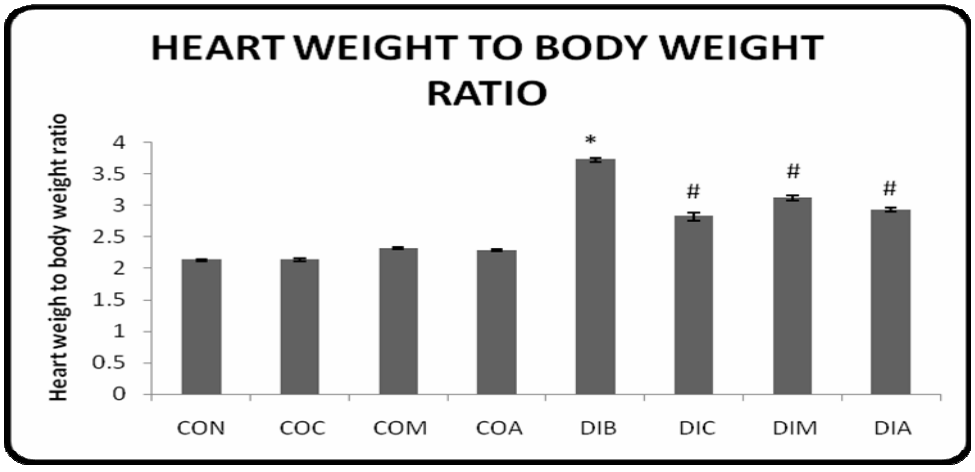


Figure 5.3.1.E

Figure 5.3.1.D. Effects of carvedilol, metoprolol and atenolol on LV & RV weight ratio.

Figure 5.3.1.E. Effects of carvedilol, metoprolol and atenolol on LV & RV weight ratio.

Each Bar contains 6 numbers of animals

* indicates significantly different from control (P<0.05)

# indicates significantly different from diabetic control group (P<0.05)

CON- control, COC- control treated with carvedilol, COM- control treated with metoprolol, COA- control treated with atenolol

DIB- Diabetic control, DIC- Diabetic treated with carvedilol, DIM- Diabetic treated with metoprolol, DIA- Diabetic treated with atenolol

5.3.2 Effect on antioxidant (Glutathione) and pro-oxidants (Lipid peroxidation)

Streptozotocin-diabetes produced a significant (P<0.05) increase in serum Malondialdehyde levels as compared to control rats. Chronic treatment with carvedilol, metoprolol and atenolol significantly (P<0.05) changes the elevated serum Malondialdehyde of diabetic rats. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the MDA Levels of control rats. (Table 5.3.2, Figure Streptozotocin-diabetes produced a significant (P<0.05) decrease in serum Glutathione levels as compared to control rats. Chronic treatment with carvedilol, metoprolol and atenolol significantly (P<0.05) changes the reduced serum Glutathione of diabetic rats. But on the other hand there is no significant change is observed in Glutathione levels of diabetic animals compared with control rats after treatment of atenolol and metoprolol. Neither of the drug produced any change in glutathione of control rats.

5.4 HEMODYNAMIC PARAMETERS

Effect on blood pressure, heart rate and rate of pressure development and decay

The mean blood pressure was significantly ($P<0.05$) increased after 8 weeks study in diabetic rats as compared to control rats. Atenolol, metoprolol and Carvedilol treatment changes the STZ induced alter blood pressure in diabetic animals. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the blood pressure of control rats (Table 5.4, Figure 5.4.A).

Heart rate was found to be significantly ($P<0.05$) lower in diabetic rats as compared to controls. Chronic treatment with carvedilol, metoprolol and atenolol in diabetic rats exhibited significant ($P<0.05$) change in heart rate as compared to diabetic control animals.

Rate of pressure development and decay was significantly ($P<0.05$) decreased in diabetic control rats. Treatment with atenolol, metoprolol and carvedilol significantly altered the rate of pressure development and decay. As well as chronic treatment with carvedilol, metoprolol and atenolol did not alter the rate of pressure development and decay of control rats.

6. DISCUSSION

Hypertension is very frequently associated with diabetic subjects (The Hypertension in Diabetes Study Group, 1993), i.e, about 50% of diabetics, irrespective of whether they are Type 1 or Type 2, are hypertensive. Hypertensive diabetics have been reported to have more cardiovascular disease when compared to normotensive diabetics. Total mortality in many epidemiological studies is two to three times higher in hypertensive diabetics when compared to that in normotensive diabetics. The total mortality in hypertensive diabetics is six to seven times higher than that in normotensive nondiabetics (UKPDS, 1998). High blood pressure (BP), obesity, and abnormal lipid profile, which often coexist with diabetes, tend to be associated with preclinical cardiovascular abnormalities and may contribute to the association of diabetes with cardiovascular events (Gu et al, 1999). Sawicki at el. (2001) reported that there are no clear evidences which gives effect of β- blockers on diabetic induced cardiovascular complications. The role of β-blocker is unclear in type 2 diabetes.

STZ produced hyperglycemia, hyperinsulinemia, hyperlipidemia, increased blood presser, increased creatinine kinase and lactate dehydrogenase enzymes and C-Reactive protein (CRP) levels, reduction in heart rate, blood presser, cardiac hypertrophy. Chronic treatment with carvedilol and metoprolol significantly ($P<0.05$) prevented STZ induced hypertension, hyperglycemia and hyperinsulinemia. Atenolol did not produce reduction in hyperglycemia and hyperinsulinemia. Carvedilol and metoprolol significantly ($P<0.05$) reduced the elevated cholesterol, very low density lipoprotein (VLDL), atherogenic index and triglyceride levels in diabetic rats and increased the lower high density lipoprotein (HDL)-cholesterol levels while atenolol showed no significant effect on lipid profile. Further, all drugs produced a significant ($P<0.05$) reduction in the elevated levels of CRP and other cardiac enzyme markers like Lactate de-hydrogenase and creatinine kinase of diabetic rats. STZ-induced bradycardia was also prevented by carvedilol and metoprolol treatment and atenolol showed no significant ($P<0.05$) effect on heart rate. All three drugs produced beneficial effect by preventing cardiac hypertrophy as evident from cardiac hypertrophy index and left ventricular hypertrophy.

Carvedilol prevent STZ induced oxidative stress but metoprolol and atenolol showed no significant ($P<0.05$) effect on antioxidant levels.

Our data suggests that carvedilol and metoprolol have beneficial effect in cardiovascular complications associated with streptozotocin (STZ) induced diabetes in neonatal rats as depicted by prevention of hyperglycemia, hyperinsulinaemia, hyperlipidemia, hypertension, bradycardia, cardiac and left ventricular hypertrophy and reduction in cardiac biomarker levels. Further, carvedilol appears to be more beneficial than metoprolol since it also prevents oxidative stress. However, atenolol shows no beneficial effect on diabetes induced cardiovascular complications.

8. REFERENCES

Aaron I. Vinik, Diabetic Cardiovascular Autonomic Neuropathy *Circulation.* 2007;115:387-397

Andrea Remuzzi, Norberto Perico, Carmen S. Amuchastegui, Barbara Malanchini, Maria Mazerska, Cristina Battaglia, Tullio Berfani, and Giuseppe Remuzzi. Short- and Long-Term Effect of Angiotensin II Receptor Blockade in Rats with Experimental Diabetes. *J. Am. Soc. Nephrol*.1993:4:40-49

Anzai, T. Yoshikawa, T. Takahashi, Y. Maekawa, T. Okabe and Y. Asakura *et al.*, Early use of beta-blockers is associated with attenuation of serum C-reactive protein elevation and favorable short-term prognosis after acute myocardial infarction, *Cardiology* 2003;99:47–53.

Arumanayagam M, Chan S, Tong S and Sanderson JE. Antioxidant Properties of Carvedilol and Metoprolol in Heart Failure: A Double-Blind Randomized Controlled Trial, Journal of Cardiovascular Pharmacology 2001;37:48-54

Asai T, Kushiro T, Fujita H and Kanmatsuse K, Different Effects on Inhibition of Cardiac Hypertrophy in Spontaneously Hypertensive Rats by Monotherapy and Combination Therapy of Adrenergic Receptor Antagonists and/or the Angiotensin II Type 1 Receptor Blocker under Comparable Blood Pressure Reduction, *Hypertension Research* 2005;28:79–87

Bakris GL et al. for the GEMINI Investigators. Metabolic effects of carvedilol vs metoprolol in patients with type 2 diabetes mellitus and hypertension: A randomized controlled trial. JAMA 2004; 292:2227-36.

Bank AJ, Kelly AS, Thelen AM, Kaiser DR, Gonzalez-Campoy JM, Effects of carvedilol versus metoprolol on endothelial function and oxidative stress in patients with type 2 diabetes mellitus. Am J Hypertens. 2007;20:777-83

Baron AD. The coupling of glucose metabolism and perfusion in human skeletal muscle. The potential role of endothelium-derived nitric oxide. *Diabetes.* 1996;45(suppl 1): S105-S109.

Batenburg WW, van E, Joep HM, Garrelds IM, Jorde U, Lamers MJ, Dekkers HW, Walther T, Kellett E, Milligan G, van K, Jorge P, Danser AH, Carvedilol-induced antagonism of angiotensin II: a matter of [alpha]1-adrenoceptor blockade. *Journal of Hypertension* 2006;24:1355-1363

Bell, D. S. H. Use of beta blockers in the patient with diabetes. *The Endocrinologist* 2003:13: 116– 123.

Bhakdi S, Toprzewski M, Klouche M, Hemmes M, Complement and atherogenesis. Binding of CRP to degraded, nonoxidised LDL enhance complement activation. Arterioscler Thromb Vasc Biol 1999;19:2348–2354

Bowie A, Owens D, Collins P, Johnson A, Tomkin GH, Glycosylated low density lipoprotein is more sensitive to oxidation: implications for the diabetic patient? Atherosclerosis 1993:102:63–67

Bristow M: Etomoxir: a new approach to treatment of chronic heart failure. *Lancet* 2000:356:1621–1622

Bristow MR: Beta-adrenergic receptor blockade in chronic heart failure. *Circulation* 2000: 101:558–569

Bristow MR: Why does the myocardium fail? Insights from basic science. *Lancet* 1998:352 :SI8_SI14

Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H: Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci USA* 1993;90:6434-6438

Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, Vlassara H: Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci USA* 1994;91:9441-9445

C. Torp-Pedersen1, C Rask-Madsen2, I Gustafsson3, F Gustafsson1, L Køber. Diabetes mellitus and cardiovascular risk: just another risk factor? *European Heart Journal Supplements* 2003:5:26—32

Carl Erik Mogensen; Giancarlo Viberti; Serge Halimi; Eberhard Ritz; Luis Ruilope; György Jermendy; Jiri Widimsky; Pinchas Sareli; Jan Taton; Juan Rull; Gürbüz Erdogan; Pieter W. De Leeuw; Arthur Ribeiro; Ramiro Sanchez; Rachid Mechmeche; John Nolan; Jana Sirotiakova; Ahmed Hamani; André Scheen; Bernhard Hess; Anton Luger; Stephen M. Thomas. Effect of Low-Dose Perindopril/Indapamide on Albuminuria in Diabetes *Hypertension.* 2003:41:1063

Carlson, L.A. and Botinger, L.E. Ischemic Heart Disease in relation to fasting values of plasma triglycerides and cholesterol. Lancet 1972;1:805-808.

Chase HP, Cooper S, Osberg I, Stene LC, Barriga K, Norris J, Eisenbarth GS, Rewers M. Elevated C-reactive protein levels in the development of type 1 diabetes. Diabetes 2003;53: 2569–2573

Chatterjee K. Congestive heart failure: what should be the initial therapy and why? *Am J Cardiovasc Drugs.* 2002; 2: 1–6.

Chattington P, Clarke D, Neithercut WD. Timed sequential analysis of creatine kinase in the diagnosis of myocardial infarction in patients over 65 years of age. J Clin Pathol. 1994;47:995-998.

Cleland SJ, Petrie JR, Small M, et al. Insulin action is associated with endothelial function in hypertension and type 2 diabetes. Hypertension 2000;35:507—11.

Cooper ME, Allen TJ, O'Brien RC, Papazoglou D, Clarke BE, Jerums G and Doyle AE, Nephropathy in model combining genetic hypertension with experimental diabetes. Enalapril versus hydralazine and metoprolol therapy. Diabetes 1990;39:1575-1579

Cruickshank, J.M. Beta blockers continue to surprise us. Eur. Heart J 2000;21:354-364

Curb JD, Pressel SL, Cutler JA, Savage PJ, Applegate WB, Black H, Camel G, Davis BR, Frost PH, Gonzalez N, Guthrie G, Oberman A, Rutan GH, Stamler J. Effect of diuretic-based antihypertensive treatment on cardiovascular disease risk in older diabetic patients with isolated systolic hypertension. Systolic Hypertension in the Elderly Program Cooperative Research Group. *JAMA.*1996;276:1886–1892

Cyrus DeSouza and Vivian Fonseca, Therapeutic targets to reduce cardiovascular disease in type 2 diabetes, *Nature reviews* 2009:8:361-367

D. K. Arulmozhi, A. Veeranjaneyulu, S. L. Bodhankar. Neonatal streptozotocin-induced rat model of Type 2 diabetes mellitus: A glance.Indian J Pharmacol 2004;36:4:217-221

Daniel I. Simon, Alvin H. Schmaier, Diabetic Platelets, Enhanced Reactivity, and Cardiovascular Risk, *Journal of the American College of Cardiology.* 2007*:*50:16

David S.H., Heart Failure, *Diabetes Care* 2003;26:2433–2441

DeBeer FC, Hind CRK, Fox KM, Allan R, Maseri A, Pepys MB. Measurement of serum C-reactive protein concentration in aspect to insulin dependant diabetes. Mol Cell Biochem 1981;37:43-61.

Dharmalingam, M., Deshpande, N., and Vidyasagar, S. Triglyceride levels and its correlation with carotid-intima-media thickness. Int. J. Diabetes Dev. Countries 2004;4:19-22.

Dillmann WH: Diabetes mellitus and hypothyroidism induce changes in myosin isoenzyme distribution in the rat heart. Do alterations in fuel flux mediate these changes? *Adv Exp Med Biol.*1986;194:469–479

Doron Aronson, Elliot J. Rayfield and James H. Chesebro. Mechanisms Determining Course and Outcome of Diabetic Patients Who Have Had Acute Myocardial Infarction.*Ann Intern Med.* 1997;126:296-306.

Doux SP, Woodley SE, Palton NJ, Wilson GL. Mechanism of nitrosourea induced β-cell damage: Alteration in DNA. Diabetes 1986;35:866-72.

Egan BM. Insulin resistance and the sympathetic nervous system. *Curr Hypertens Rep.* 2003;5:247-254.

Eichhorn EJ, Bristow MR: Medical therapy can improve the biological properties of the chronically failing heart: a new era in the treatment of heart failure. *Circulation.*1986;94:2285–2296

Estacio RO, Jeffers BW, Hiatt WR, Biggerstaff SL, Gifford N, Schrier RW, The effect of nisoldipine as compared with enalapril on cardiovascular outcomes in patients with non-insulin-dependent diabetes and hypertension*. N Engl J Med* 1998;338:645–652

Factor SM, Minase T, Sonnenblick EH: Clinical and morphological features of human hypertensive-diabetic cardiomyopathy. *Am Heart J* 1980;99:446–458

Festa A, D'Agostino R Jr, Hales CN, Mykkanen L, Haffner SM: Heart rate in relation to insulin sensitivity and insulin secretion in nondiabetic subjects. *Diabetes Care* 2000;23:624 –628

Feuerstein GZ and Ruffolo JR, Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection. European Heart Journal 2000;16:38-42.

Fleischmann EH, Schmieder RE. Are all antihypertensive drug classes equal in reducing left ventricular hypertrophy? *Curr Cardiol Rep.* 2002;4:474–8

Frangogiannis NG, Smith CW, Entman ML: The inflammatory response in myocardial infarction. Cardiovasc Res 53;31–47, 2002

Fukunaga Y, Itoh H, Doi K, Tanaka T, Yamashita J, Chun TH, Inoue M, Masatsugu K, Sawada N, Saito T, Hosoda K, Kook H, Ueda M, Nakao K: Thiazolidinediones, peroxisome proliferatoractivated receptor gamma agonists, regulate endothelial cell growth and secretion of vasoactive peptides. *Atherosclerosis* 2001;158:113–119

Funakawa S. Renin angiotensin system and prostacyclin biosynthesis in STZ diabetic rats. Eur J Pharmacl 1983;94:27–33

G. Mancia. The association of hypertension and diabetes: prevalence,cardiovascular risk and protection by blood pressure reduction. Acta Diabetol 2005;42:S17–S25

Ganguly PK, Pierce GN, Dhalla KS, Dhalla NS: Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. *Am J Physiol* 1983;244:E528-E535

Gavras H, Kremer D, Brown JJ, Gray B, Lever AF, MacAdam RF, Medina A, Morton JJ, Robertson JI: Angiotensin and norepinephrine-induced myocardial lesions: experimental and clinical studies in rabbits and man. *Am Heart J* 1975;89:321–332

Genda A, Mizuno S, Nunoda S, Nakayama A, Igarashi Y, Sugihara N, Namura M, Takeda R, Bunko H, Hisada K: Clinical studies on diabetic myocardial disease using exercise testing with myocardial scintigraphy and endomyocardial biopsy. *Clin Cardiol* 1986;9:375–382

George L. Bakrisa, The rationale and design of the Glycemic Effects in Diabetes Mellitus Carvedilol–Metoprolol Comparison in Hypertensives (GEMINI) trial, *Journal of Diabetes and Its Complications* 2005:19:74– 79

Gilbert, E.M., Abraham, W.T., Olsen, S., Hattler, B., White, M., Mealy, P., et al. Comparative hemodynamic, left ventricular functional and antiadrenergic effect of chronic treatment with metoprolol versus carvedilol in the failing heart. Circulation 1996;94: 2817-2825

Giugliano D, Acampora R, Marfella R, et al. Metabolic and cardiovascular effects of carvedilol and atenolol in non-insulin-dependent diabetes mellitus and hypertension. A randomized, controlled trial. *Ann Intern Med.* 1997;126:955-959.

Giugliano D, Acampora R, Marfella R, Rosa ND, Ziccardi P, Ragone R, Angelis LD, and D'Onofrio F, Metabolic and Cardiovascular Effects of Carvedilol and Atenolol in Non-Insulin-Dependent Diabetes Mellitus and Hypertension- A Randomized, Controlled Trial, *Ann Intern Med.* 1997;126:955-959

Gorden T, Kannel WB. Premature mortality from coronary heart disease. The Framingham study. JAMA 1971;215:1617–1625

Grines CL, Topol EJ, Califf RM, Stack RS, George BS, Kereiakes D, et al. Prognostic implications and predictors of enhanced regional wall motion of the noninfarct zone after thrombolysis and angioplasty therapy of acute myocardial infarction. TheTAMI Study Groups. *Circulation* 1989;80:245-53

Grossman E, Shemesh J, Shamiss A, Thaler M, Carroll J, Rosenthal T. Left ventricular mass in diabetes. Arch Int Med 1992;152:5

Gu, K., Cowie, C. C., & Harris, M. I. Diabetes and decline in heart disease mortality in US adults. Journal of the American Medical Association 1999;281:1291– 1297.

Guideline Subcommittee of the Japanese Society of Hypertension (JSH). Guidelines for the management of hypertension. 2000:1 –125.

Hagar HH, Folic acid and vitamin B12 supplementation attenuates isoprenaline-induced myocardial infarction in experimental hyperhomocysteinemic rats. Pharmacol Res 2002;46(3): 213–219

Hanada K, Asari K, Saito M, Kawana J, Mita M, Ogata H, Comparison of pharmacodynamics between carvedilol and metoprolol in rats with isoproterenol-induced cardiac hypertrophy: effects of carvedilol enantiomers., , Eur J Pharmacol. 2008 Jul 28;589(1-3):194-200.

Haneda T, Ogawa Y, Kato J, Matsuhashi H, Morimoto H, Honda H, Takenaka T, Tanazawa S, Kataoka R, Kikuchi K. Effect of celiprolol on cardiac hypertrophy in hypertension Hypertens Res. 2000;23(5):467-74.

Hansson, L., Zanchetti, A., Carruthers, S. G., Dahlof, B., Elmfeldt, B., Julius, S., et al. for the HOT Study Group. Effects of intensive blood-pressure lowering and acetylsalicylic acid in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomized trial. *Lancet* 1998:351:1755–1762.

Hauf-Zachariou U, Widmann L, Zulsdorf B, et al. A double-blind comparison of the effects of carvedilol and captopril on serum lipid concentrations in patients with mild to moderate essential hypertension and dyslipidaemia. *Eur J Clin Pharmacol.* 1993;45:95-100.

Hausdorff, W.P., Caron, M.G., and Lefkowitz, R.J. Turning off the signal: desensitization of beta-adrenergic receptor function. FASEB J. 1990;4: 2881-2889. PMID: 2165947.

Heart Outcomes Prevention Evaluation (HOPE) Study Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet*.2000: 355:253–259

Helmy M. Siragy, ADVANCE in Management of Vascular Complications of Diabetes, *Lancet* 2007;370: 829– 840.

Hermenegildo C, Raya A, Roma J, et al: Decreased glntathione peroxidase activity m sciatic nerve of alloxan-induced diabetic and its correlation with blood glucose levels. Neurochem Res. 1993;18:893- 896

Howard-Alpe GM, Sear JW, Foex P Methods of detecting atherosclerosis in non-cardiac surgical patients; the role of biochemical markers. *Brit J Anaesth* 2006;97(6):758–769

Huang E, Kuo W, Chen Y, Chen T, Chang M, Lu M, Tzang B, Hsu H, Huang C, Lee S Homocysteine and other biochemical parameters in type 2 diabetes mellitus with different diabetic duration or diabetic retinopathy. *Clinica Himica Acta* 2006;366(1–2):293–298

Huang H , Shan J, Pan XH, Wang HP, Qian LB and Xia Q, Carvedilol improved diabetic rat cardiac function depending on antioxidant ability, Diabetes Research and Clinical Practice 2007;75: 7-13

Ide, M., Katayama, S., Tanaka, K., Itabashi, A., Kawazu, S., & Ishii, J. Effect of alpha1-blockade on diminished forearm blood flow in diabetics. *Diabetes Research and Clinical Practice* 1991;12:157– 162.

Itskovitz, H.D. $Alpha_1$ blockers: Safe effective treatment for hypertension. Postgrad. Med. 1991;89: 89-112. PMID: 1674822.

Jacob S, Rett K, Wicklmayr M, et al. Differential effect of chronic treatment with two beta-blocking agents on insulin sensitivity: the carvedilol-metoprolol study. *J Hypertens.* 1996;14:489-494.

Johnsson. Influence of metoprolol and propranolol on hemodynamic effects induced by adrenaline and physical work. *Acta Pharmacol Toxicol (Copenh).* 1975;36 (Suppl V):59-68.

Joynt KE, Gattis WA, Hasselblad V, Fuzaylov SY, Serebruany VL and Gurbel PA *et al.*, Effect of angiotensin-converting enzyme inhibitors, beta blockers, statins, and aspirin on C-reactive protein levels in outpatients with heart failure, *Am J Cardiol* 2004;93:783–785.

Kaiser P. Physical performance and muscle metabolism during beta-adrenergic blockade in man (thesis). Acta PhysiolScand 1984;suppl 536:1-53.

Kamble MM, Vaidya SM, Effect of antihypertensive drugs on cardiac enzymes in hypertension with myocardial infarction in NIDDM. Indian. *J. Clin. Biochem* 2002;17(2): 60 – 63.

Kannel WB, McGee DL, Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care 1979;2:120–126

Kashiwabara, H., Inaba, M., Maruno, Y., Morita, T., Awata, T., Negishi, K., et al. Insulin levels during fasting and the glucose tolerance test and Homa's index predict subsequent development of hypertension. *Journal of Hypertension* 2000;18:83– 88.

Kersten JR, Brooks LA, Dellsperger KC. Impaired microvascular response to graded coronary occlusion in diabetic and hyperglycemic dogs. *Am J Physiol*. 1995;268(4 Pt 2):H1667-74.

Kumar A, Cannon CP. Acute coronary syndromes: diagnosis and management, part I. Mayo Clin Proc. 2009;84:917-938.

Lager I, BLOHME G, SMITH. Effect of cardioselective and nonselective β-blockade on the hypoglycemic response in insulin-dependent diabetics. *Lancet.* 1979;1:458-62.

Lager I, Blohme G, Smith. Effect of cardioselective and nonselective β-blockade on the hypoglycemic response in insulin-dependent diabetics. *Lancet.* 1979*;*1*:*458-*62.*

Lanza GA, Pitocco D, Navarese EP, Sestito A, Sgueglia GA, Manto A,Infusino F, Musella T, Ghirlanda G, Crea FAssociation between cardiac autonomic dysfunction and inflammation in type 1 diabetic patients: effect of beta-blockade*. Eur Heart J* 2007*;*28*:*814-820*.*

Lillioia S, Young AA, Culter CL, et al. Skeletal muscle capillary density and fibre type are possible determinants of in vivo insulin resistance in man. J Clin Invest 1984;suppl 536:1-53.

Lithell H, Lindgarde F, Heilsing K, et al. Body weight, skeletal muscle morphology and enzyme activities in relation to fasting serum insulin concentration and glucose tolerance in 48-year-old men. Diabetes 1981;30: 19-25.

Liu JC, Hsu FL, Tsai JC, Chan P, Liu JY, Thomas GN, Tomlinson B, Lo MY, Lin JY. Antihypertensive effects of tannins isolated from traditional Chinese herbs as nonspecific inhibitors of angiotensin converting enzyme. Life Sci 2003;73(12):1543-55.

Löwel H, Koenig W, Engel S, Hörmann A, Keil U. The impact of diabetes on survival after myocardial infarction: can it be modified by drug treatment? *Diabetologia* 2000:43:218–26.

Lowes BD, Abraham WT, Minobe WA, Gilbert EM, Roden RL, Bristow MR: Dynamic changes in the expression of contractility-regulating genes in the failing human heart associated with improvement or deterioration in ventricular systolic function.*Circulation* 1998;98 (Suppl. 1):I-361

Lown JN, Mc Laughlin LW, Chang Y. Mechanisms of action of 2-halo ethyl nitrosoureas on DNA and its relations to their anti-leukemic properties. Bioorg Chem 1979;7:97-110.

Malmberg K, Herlitz J, Hjalmarson A, Ryden L, Effects of metoprolol on mortality and late infarction in diabetics with suspected acute myocardial infarction. Retrospective data from two large studies. *Eur Heart J.*1989;10:423–428

Malmberg K, Ryde´n L, Hamsten A, Herlitz J, Waldenstro¨m A, Wedel H. Effects of insulin treatment on cause specific one-year mortality and morbidity in diabetic patients with acute myocardial infarction. Eur Heart J 1996;17:1337–1334.

Mandinov L, Eberli FR, Seiler C, Hess OM. Diastolic heart failure. *Cardiovasc Res.* 2000; 45:813–825.

Mann DL, Kent RL, Parsons B, Cooper G: Adrenergic effects on the biology of the adult *mammalian* cardiocyte. *Circulation* 1992;85:790–804

Markku Laakso. Hyperglycemia and Cardiovascular Disease in Type 2 Diabetes. *DIABETES*. 1999;48:197-184

Mauras N, Blizzard RM, Thorner MO, Rogol AD. Selective betaI-adrenergic receptor blockade with atenolol enhances growth hormone releasing hormone and mediated growth hormone release in man. Metabolism 1987;36:369-72.

Mayer B, Holmer SR, Hengstenberg C, Lieb W, Pfeifer M and Schunkert M, Functional improvement in heart failure patients treated with beta blockers is associated with a decline of cytokine levels, *Int J Cardiol* 2005;**103**:182–186

Meigs JB, Singer DE, Sullivan LM, Dukes KA, D'Agostino RB, Nathan DM, Wagner EH, Kaplan SH, Greenfield S: Metabolic control and prevalent cardiovascular disease in non-insulin-dependent diabetes mellitus (NIDDM): the NIDDM Patient Outcome Research team. *Am J Med* .1997;102:38–47

Messerli FH, Grossman E, Goldbourt U. Are beta-blockers efficacious as first-line therapy for hypertension in the elderly? A systematic review. *JAMA.* 1998;279:1903–7.

Mitchell LB, Powell JL, Gillis AM, et al. Are lipid-lowering drugs also antiarrhythmic drugs? An analysis of the Antiarrhythmics Versus Implantable Defibrillators (AVID) trial. *J Am Coll Cardiol* 2003;42:81-87.

Mizushige K, Yao L, Noma T, Kiyomoto H, Yu Y, Hosomi N, Ohmori K, Matsuo H, Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulinresistant prediabetic stage of a type II diabetic rat model. Circulation 2000;101:899–907

Morishima I, Sone T, Tsuboi H, Kondo J, Mukawa H, Kamiya H, Hieda N, Okumura K: Plasma C-reactive protein predicts left ventricular remodeling and function after a first acute anterior wall myocardial infarction treated with coronary angioplasty: comparison with brain natriuretic peptide. Clin Cardiol 25;112–116, 2002
Mortality prediction in diabetic patients with myocardial infarction: experiences from the DIGAMI study. *Cardiovascular Research* 1997;34:248–253

Murray DR, Prabhu DS and Chandrasekar B, Chronic β-adrenergic stimulation induces myocardial proinflammatory cytokine expression, *Circulation* 2000;101:2338–2341.

Nagatomo Y, Yoshikawa T, Kohno T, Yoshizawa A , Anzai T , Meguro T, Satoh T and Ogawa S Effects of β-Blocker Therapy on High Sensitivity C-Reactive Protein, Oxidative Stress, and Cardiac Function in Patients With Congestive Heart Failure. Journal of Cardiac Failure, 2007;13:365-371

Östman J, Arner P, Haglund K, Julin-dannfelt A, Novae J, Wennlund A, *A cardio-selective beta-blocker (metoprolol) in hypertensive insulin-dependent diabetics. Acta Med Scand (Suppl). 1980;639:29-32.*

Ouvina SM, La Greca RD, Zanaro NL, et al. Endothelial dysfunction, nitric oxide and platelet activation in hypertensive and diabetic type II patients. Thromb Res 2001;102:107—14.

P. T. Sawicki, A. Siebenhofer. Beta-Blockers and Diabetes Mellitus. *J Clin Basic Cardiol* 2001: 4: 17

Panchal AR, Stanley WC, Kerner J, Sabbah HN: Beta-receptor blockade decreases carnitine palmitoyl transferase I activity in dogs with heart failure. *J Card Fail.*1998:4:121–126

Pedersen F, Ren S, Rasmussen L, Kolendorf K and Christiansen E, The effect of alprenolol on serum myoglobin levels in acute myocardial infarctio. Sc*andinavian Journal of Clinical and Laboratory Investigation* 1984; 44:649 - 654

Perin PC, Maule S, Quadri R. Sympathetic nervous system, diabetes, and hypertension. *Clin Exp Hypertens.* 2001; 23: 45–55.

Piccini JP, Klein L, Gheorghiade M, Bonow RO. New insights into diastolic heart failure: role of diabetes mellitus. *Am J Med.* 2004; 116 (suppl 5A): 64S–75S.

Pietila KO, Harmoinen AP, Jokiniitty J, Pasternack AI: Serum C-reactive protein concentration in acute myocardial infarction and its relationship to mortality during 24 months of follow-up in patients under thrombolytic treatment. *Eur Heart J* 17;1345–1349, 1996

Poole-Wilson PA, Swedberg K, Cleland JGF, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol or Metoprolol European Trial (COMET); randomized controlled trials. *Lancet* 2003;362:7-13.

Portha B, Blondel O, Serradas P, Mc Evoy R, Girox MH, Kergoat, et al. The rat models of non-insuin dependant diabetes induced by neonatal streptozotocin. *Diabete Metab* 1989;15:61-75.

Prabhu DS, Chandrasekar B, Murray DR and Freeman GF, β-Adrenergic blockade in developing heart failure: effects on myocardial inflammatory cytokines, nitric oxide, and remodeling, *Circulation* 2000;101:2103–2109.

Pradhan A, Manson J, Rifai N, Buring J, Ridker P. Creactive protein, interleukin-6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001;286:327–334

Rabasseda, X, Carvedilol: An effective antihypertensive drug with antiischemic/antioxidant cardioprotective properties**,** *Drugs Today* 1998;34(11):905

Rani SH, Rao DV, Prakash SM, Jyothy A. Serum Adenosine deaminase activity and C-reactive protein levels in unstable angina. *Indian J Human Genet* 1994;9(1):17–20

Raymond O. Estacio, Barrett W. Jeffers, Nancy Gifford, RN, Robert W. Schrier. Effect of Blood Pressure Control on Diabetic Microvascular Complications in Patients With Hypertension and Type 2 Diabetes, *Diabetes Care.*2000;23:B54–B64

Refsgaard J, Thomsen C, Andreasen F, et al. Carvedilol does not alter the insulin sensitivity in patients with congestive heart failure. *Eur J Heart Fail.* 2002;4:445-453.

Romeo F, Li D, Shi M and Mehta JL, Carvedilol prevents epinephrine-induced apoptosis in human coronary artery endothelial cells, *Cardiovascular Research* 1999;45;3788-794

S. Anderson. Antihypertensive Therapy in Experimental Diabetes. *J. Am. Soc. Nephrol.* 1992; 3:S86-S90

Saenger AK, Jaffe AS. The use of biomarkers for the evaluation and treatment of patients with acute coronary syndromes. *Med Clin North Am*. 2007;91:657-681.

Sakamoto K, Yamasaki Y, Nanto S, Shimonagata T, Morozumi T, Ohara T, Takano Y, Nakayama H, Kamado K, Nagata S, Kusuoka H, Nishimura T, Hori M. Mechanism of impaired left ventricular wall motion in the diabetic heart without coronary artery disease. *Diabetes Care.* 1998;21:2123–2128.

Satia, M.C., Shukla M. L., Gandhi, T.P., and Goyal R.K. Prevalence of hypertension and comparative evaluation of four antihypertensive monotherapy in Indian NIDDM hypertensive patients. *Indian J. Hypertens* 1997;2: 17-27.

Savarese JJ, Berkowitz BA. Beta adrenergic receptors decrease in diabetic rat hearts. Life Sci 1970;25:2075–2078

Savarese, J.J., and Berkowitz, B.A. Beta adrenergic receptors decrease in diabetic rat hearts. *Life sci.* 1979;25: 2075-2078. doi: 10.1016/0024-3205(79)90200-5. PMID: 231718.

Sekiguchi K, Li X, Coker M, Flesch M, Barger PM, Sivasubramanian N, Mann DL. Cross-regulation between the renin-angiotensin system and inflammatory mediators in cardiac hypertrophy and failure. *Cardiovasc Res.* 2004;63:433–442.

Sharp RP, Impact of Carvedilol on the Serum Lipid Profile. *The Annals of Pharmacotherapy* 2008;42:564-571

Shigehiro Katayama; Munemichi Inaba. Importance of blood pressure control in patients with diabetes mellitus Journal of Diabetes and Its Complications. *Hypertention* 2002:16:87– 91

Sleight P. Beta blockade early in acute myocardial infarction. *Am J Cardiol* 1987;60:6A–10A

Stamler J, Vaccaro O, Neaton JD, Wentworth D Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 1993;16:434–444

Steinbrecher UP, Witztum JL: Glucosylation of low-density lipoproteins to an extent comparable to that seen in diabetes slows their catabolism. *Diabetes* 1984, 33:130-134

Stone PH, Muller JE, Hartwell T, York BJ, Rutherford JD, Parker CB, Turi ZG, Strauss HW, Willerson JT, Robertson T: The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis: the MILIS Study Group. *J Am Coll Cardio.l.*1989*;*14:49 –57

Takahashi T, Anzai T, Yoshikawa T, Maekawa Y, Asakura Y and Satoh T *et al.*, Serum C-reactive protein elevation in left ventricular remodeling after acute myocardial infarction—role of neurohormones and cytokines, *Int J Cardiol* 2003;88:257–265.

Taskinen M. Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. Diabetes 1992;41:12–17

The effects of tissue plasminogen activator, streptokinase, or both on coronary-artery patency, ventricular function, and survival after acute myocardial infarction. The GUSTO Angiographic Investigators. *N Engl J Med* 1993;329: 1615-22

The Hypertension in Diabetes Stydy Group. Hypertension in Diabetes Study (HDS): 1. Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with

risk factors for cardiovascular and diabetic complications. *Journal of Hypertension*. 1993;11:309– 317.

Tölg R , Witt M, Schwarz B, Kurz T, Kurowski V, Hartmann F, Geist V and Richardt G, Comparison of carvedilol and metoprolol in patients with acute myocardial infarction undergoing primary coronary intervention —The PASSAT Study. *Clinical Research in Cardiology* 2006;95:31-41

Tomlinson, K.C., Gardiner, S. M., Herdes, R.A., and Binnet, T. Functional consequences of streptozotocin induced diabetes mellitus, with particular reference to the cardiovascular system. *Pharmacol. Rev.* 1992;44: 103-150. PMID: 1557425.

Tooke JE. Microvascular function in human diabetes. A physiological perspective. *Diabetes* 1995:44:721–726

Tooke, J. E., & Goh, K. L. Endothelinopathy precedes type 2 diabetes. *Diabetes Care.*1998;12:2047– 2049.

UK Prospective Diabetes Study Group. Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. *BMJ.*1998;317:713–720

UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ*. 1998;317:703–713.

UK Prospective Diabetes Study Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*.1998;352:837–853

Umans JG, Levi R. Nitric oxide in the regulation of blood flow and arterial pressure. *Annu Rev Physiol.* 1995;57:771-90.

Van Herwaarden CLA, Binkhorst RA, Fennis JFM, Laar AV, *Effects of adrenaline during treatment with propranolol and metoprolol. Br Med J.* 1977;1:1029. *Letter.*

Van Herwaarden CLA, Binkhorst RA, Fennis JFM, Laar AV, Effects of adrenaline during treatment with propranolol and metoprolol. *Br Med J.* 1977*;*1*:*1029*. Letter.*

Vittorio Palmieri, Jonathan N. Bella, Donna K. Arnett, Jennifer E. Liu, Albert Oberman, Min-Yan Schuck, Dalane W. Kitzman, Paul N. Hopkins, Derek Morgan, D.C. Rao, Richard B. Devereux. Effect of Type 2 Diabetes Mellitus on Left Ventricular Geometry and Systolic Function in Hypertensive Subjects: Hypertension Genetic Epidemiology Network (HyperGEN) Study. *Circulation* 2001;103:102-107

Weber KT, Brilla CG: Pathological hypertrophy and cardiac interstitium: fibrosis and renin-angiotensin-aldosterone system. *Circulation.*1991;83:1849–1865

Wei S,, Louis, Chow LT and Sanderson JE, Effect of carvedilol in comparison with metoprolol on myocardial collagen postinfarction *J Am Coll Cardiol*, 2000; 36:276-281

Weidmann, P., Uehlinger, D. E., and Gerber, A. Antihypertensive treatment and serum lipoproteins. *J. Hypertens*. 1985;3: 295-306.

Williamson JR, Kilo C, Capillary basement membrane thickening and diabetic microangiopathy. *Diabetes* 1976;25:925–927

Wolff SR and Dean RT: Glucose autoxidation and protein modification. Biochem J 1987; 245:243-250, Low PA, Nickander KK: Oxygen free radical effects in sciatic nerve in experimental diabetes. *Diabetes* 1991;40:873-877

Xu DL, He H, Zeng P, Lai W, Ren H, Effects of Carvedilol and Metoprolol on Plasma advanced Glycation End Products (AGE) and Advanced Oxidation Protein Products (AOPP) in Patients with Chronic Heart Failur, *Circulation* 2006;114:II_571

Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G: Effects of anangiotensin converting- enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators , *N Engl J Med* 2000;342:748

Zaninetti D, Greco-Perotto R, Jaenrenaud B. Heart glucose transport and transporters in rat heart: regulation by insulin, workload and glucose. *Diabetologia*. 1988;31:108-13.

Zile MR, Brutsaert DL, New concepts in diastolic dysfunction and diastolic heart failure: part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 2002;105:1387–1393