Review of Literature
3. REVIEW OF LITERATURE

Diabetes mellitus was first described in India in the ancient texts of Charaka and Sushruta (1500 BCE). Since then, the disease has gradually evolved into a major public health problem. The prevalence of diabetes mellitus is growing rapidly. It is estimated that globally the number of adults affected with diabetes will increase from 135 million in 1995 to 300 million by 2025 (King et al., 1998). Diabetes has emerged as a major healthcare problem in India. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. It is estimated that every fifth person with diabetes will be an Indian. Due to these sheer numbers, the economic burden due to diabetes in India is amongst the highest in the world. The real burden of the disease is however due to its associated complications which lead to increased morbidity and mortality (Anjana et al., 2011).

The vascular complications are the leading causes of mortality and morbidity in diabetic patients. Diabetes doubles the risk of cardiovascular disease (Garcia et al., 1974). The main "macrovascular" diseases (related to atherosclerosis of larger arteries) are ischemic heart disease (angina and myocardial infarction), stroke and peripheral vascular disease. Diabetes also damages the capillaries causing microangiopathy (Boudina and Dale Abel, 2007). Diabetic retinopathy, which affects blood vessel formation in the retina of the eye, can lead to visual symptoms, reduced vision, and potentially blindness. Diabetic nephropathy, the impact of diabetes on the kidneys, can lead to scarring changes in the kidney tissue, loss of small or progressively larger amounts of protein in the urine, and eventually chronic kidney disease requiring dialysis. Diabetic neuropathy is the impact of diabetes on the nervous system, most commonly causing numbness, tingling and pain in the feet and also increasing the risk of skin damage due to altered sensation. Together with vascular disease in the legs, neuropathy contributes to the risk of diabetes-related foot problems (such as diabetic foot ulcers) that can be difficult to treat and occasionally require amputation.

Patients with diabetes are characterized by an increased likelihood of heart failure, largely reflecting the contribution of diabetes to coronary artery disease and its
association with hypertension. Over the last three decades, a number of epidemiological, autopsies, animal, and clinical studies have proposed the presence of diabetic heart disease as a distinct clinical entity (Boudina and Dale Abel, 2007). However, the existence of diabetic heart disease or cardiomyopathy—referring to myocardial disease in diabetic subjects that cannot be ascribed to hypertension, coronary artery disease, or any other known cardiac disease—has remained controversial.

The Framingham study demonstrated the increased incidence of congestive heart failure (HF) in diabetic males (2.4:1) and females (5:1) independent of age, hypertension, obesity, CAD and hyperlipidemia (Kannel et al., 1976). Other prospective studies also show that diabetic patients have a significantly increased lifetime risk of developing HF, and increased mortality from both Q-wave and non-Q-wave myocardial infarction (Herlitz et al., 1988). This suggests that there is an additional insult to diabetic myocardium which predisposes it to more extensive damage and subsequent failure. Bertoni et al (2003) have shown a link between idiopathic cardiomyopathy and diabetes. In contrast with the 4–6% prevalence of diabetes in the community, the overrepresentation of diabetic patients in HF trials such as SOLVD (Studies of Left Ventricular Dysfunction; 26%, Shindler et al., 1996), ATLAS (Assessment Trial of Lisinopril and Survival; 19%, Ryden et al., 2000) and V-HeFT II (Vasodilator-Heart Failure Trial II; 20%, Cohn et al., 1991) attests to the increased prevalence of this condition among diabetic patients.

Diabetic vascular complications result from imbalances caused by increases in the toxic effects of systemic metabolic abnormalities such as hyperglycemia, dyslipidemia, and hypertension, and reductions in the regenerative effects of endogenous protective factors such as insulin, vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), nitric oxide (NO), and antioxidant enzymes (Jeong and King, 2011, Fig. 3.1). In the past, many studies on the mechanisms of diabetic complications have focused on the mechanisms by which hyperglycemia might lead to the chronic vascular complications via the formation of toxic metabolites such as oxidants and advanced glycosylated products. These mechanisms include increases in oxidative stress, persistent activation of protein kinase C (PKC) and other signaling pathways, increased
sorbitol concentrations through the aldose reductase pathway, the elevated formation of advanced glycosylation end products, and increased flux through the hexosamine pathway (Brownlee, 2001). However, few studies have evaluated the importance of endogenous protective factors or the inhibitory effects of hyperglycemia in neutralizing these protective factors during the initiation and progression of diabetic complications.

![Diagram](image)

**Figure 3.1:** Diabetes induces an imbalance between toxic and protective factors to cause complications. FFA, free fatty acid; AGE, advanced glycosylated end product; ROS, reactive oxygen species; PKC, protein kinase C; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; APC, activated protein C.

Alterations in myocardial structure and function occur in the late stage of diabetes (Cai and James Kang, 2001). These chronic alterations are believed to result from acute cardiac responses to suddenly increased glucose levels at the early stage of diabetes. Oxidative stress, induced by reactive oxygen and nitrogen species derived from hyperglycemia, causes abnormal gene expression, altered signal transduction, and the activation of pathways leading to programmed myocardial cell deaths. The resulting myocardial cell loss thus plays a critical role in the development of diabetic cardiomyopathy.
DIABETIC CARDIOMYOPATHY

Diabetes mellitus can affect cardiac structure and function in the absence of changes in blood pressure and coronary artery disease, a condition called diabetic cardiomyopathy. This term was introduced 30 years ago by Rubler et al., 1972, who described 4 diabetic patients with congestive heart failure and normal coronary arteries. Since then, diabetic cardiomyopathy has been defined as ventricular dysfunction that occurs independently of coronary artery disease and hypertension.

Diabetic cardiomyopathy refers to a disease process which affects the myocardium in diabetic patients causing a wide range of structural abnormalities eventually leading to left ventricular hypertrophy (LVH) and diastolic and systolic dysfunction or a combination of these (Hayat et al., 2004). The concept of diabetic cardiomyopathy is based upon the idea that diabetes is the factor which leads to changes at the cellular level, leading to structural abnormalities as outlined above. We know that diabetic patients are at increased risk of hypertension and coronary artery disease (CAD); however, the idea of the existence of a diabetic cardiomyopathy suggests that changes can occur and be detected without the presence of these other factors. Therefore patients with hypertension and CAD may well have myocardial changes related to these disease processes, but a specific cardiomyopathy may also affect the myocardium secondary to diabetes causing a synergistic adverse effect as seen with a combination of diabetes and hypertension (Hayat et al., 2004). Diabetic cardiomyopathy can be subclinical or apparent depending on the presence of symptoms and signs. There appears hyperglycemia, hyperlipidemia and increased ROS (reactive oxygen species) induce alterations in downstream transcription factors which result in changes in gene expression, myocardial substrate utilization, myocyte growth, endothelial function and myocardial compliance to be a long subclinical course in most patients before the development of symptoms (Amour and Kersten, 2008). The Doppler echocardiographic findings in the STZ-induced diabetic animals showed evidence of a cardiomyopathy characterized by eccentric hypertrophy, abnormal LV filling and systolic dysfunction. In vivo and in vitro hemodynamic data revealed impaired systolic and diastolic function (Levy et al., 1990).
STRUCTURAL CHANGES IN DIABETIC CARDIOMYOPATHY

Left ventricular hypertrophy (LVH)

LVH is often defined as the upper 5% of the distribution of LV mass in the population. Although there is no clear consensus on the cut-off values used to define LVH, the 2003 ESC (European Society of Cardiology) guidelines proposed that LVH on echocardiography should be defined by an LV mass >125 g/m² for men and 110 g/m² for women. Alternatively, the 12-lead ECG remains a useful qualitative screening technique for LVH. Framingham study showed that the risk factor-adjusted relative risk of cardiovascular disease was 1.49 for each increment of 50 g/m in LV mass for men and 1.57 for women (Levy et al., 1990). The presence of LVH has been linked with increased markers of systemic inflammation [fibrinogen and CRP (C-reactive protein)] and microalbuminuria and, in a study of 1299 Type II diabetic patients, increased albuminuria was a marker of endothelial damage and increased atherothrombotic risk (Palmieri et al., 2003).

FUNCTIONAL CHANGES IN DIABETIC CARDIOMYOPATHY

Systolic dysfunction

The definition of systolic dysfunction is impairment in the ability of the heart to eject blood. Although the principle hallmark of systolic dysfunction is a depressed LV ejection fraction, recent studies have shown that standard 2D (two-dimensional) echocardiography may actually miss subtle LV dysfunction, since circumferential LV function is assessed and longitudinal function overlooked (Petrie et al., 2002). In the context of diabetic cardiomyopathy, systolic dysfunction occurs late, often when patients have already developed significant diastolic dysfunction. The prognosis in patients with depressed systolic dysfunction is poor with an annual mortality of 15-20%.

Diastolic dysfunction

Diastole is the time period where the myocardium is no longer generating force and subsequently returns to an unstressed length and force. Diastolic dysfunction occurs
when there is prolongation and slowing of this process. Diastolic function can be defined by examining trans-mitral and trans-pulmonary flow rates (Ommen and Nishimura, 2003). An important limitation in using trans-mitral flow alone as a marker of diastolic dysfunction is related to the rise in left atrial pressure which occurs with diastolic dysfunction, leading to a pseudo-normalization of the mitral inflow pattern. Thus, although there is progressive diastolic dysfunction, the mitral inflow pattern appears normal. Furthermore, it has been shown that diastolic dysfunction is not just a defect in active relaxation, but also in passive stiffness of the left ventricle (Zile et al., 2004). In the course of diabetic cardiomyopathy, a spectrum of myocardial abnormalities develop and progress which include LVH and diastolic and systolic dysfunction. It is thus incumbent upon clinicians to identify these abnormalities, since early detection and appropriate treatment can prevent worsening of this condition to overt HF.

MOLECULAR BASIS FOR DIABETIC CARDIOMYOPATHY

Hyperglycaemia, hyperlipidaemia and increased ROS (reactive oxygen species) induce alterations in downstream transcription factors which result in changes in gene expression, myocardial substrate utilization, myocytes growth, endothelial function and myocardial compliance. These effects are summarized in Table 3.1.

Potential contributors to the development of diabetic cardiomyopathy are shown in figure 3.2. Increased free fatty acid (FFA) activates PPAR- signaling, leading to the increased transcription of many genes involved in FA oxidation (Boudina and Dale Abel, 2007). Increased FA oxidation leads to the generation of ROS at the level of the electron transport chain. ROS, which also can be generated by extramitochondrial mechanisms such as NADPH oxidase, plays a critical role in several pathways involved in the pathogenesis of diabetic cardiomyopathy, including lipotoxicity, cell death, and tissue damage, as well as mitochondrial uncoupling and reduced cardiac efficiency.
Table 3.1: Summary of the major molecular abnormalities and their consequence in the pathogenesis of diabetic cardiomyopathy.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Mechanism</th>
</tr>
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<tbody>
<tr>
<td>Hyperglycemia</td>
<td>Excess AGE and ROS formation with deactivation of NO, myocardial collagen deposition and fibrosis (Singh et al., 2001; Young et al., 2002).</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Impaired glycolysis, pyruvate oxidation, lactate uptake results in apoptosis, and perturbation of myocardial bioenergetics and contraction/relaxation coupling (Zhou et al., 2000).</td>
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<tr>
<td>PKC</td>
<td>Activation of DAG/PKC signal transduction pathway leads to reduction in tissue blood flow, increased vascular permeability, alterations in neovascularization and enhanced extracellular matrix deposition (Way et al., 2001).</td>
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<tr>
<td>RAS</td>
<td>Cardiomyocyte hypertrophy and apoptosis (Kajstura et al., 2001). Aldosterone-induced fibrosis Myofibroblast growth with interstitial and focal perivascular accumulation of collagen.</td>
</tr>
<tr>
<td>HIF-1/VEGF</td>
<td>HIF-1α activation via hypoxia/free radicals induces angiopoietin, PGF, PDGF-β and VEGF (Kelly et al., 2003) but, in diabetes, VEGF and its receptors, VEGF-R1 and VEGF-R2, are decreased significantly, leading to impaired angiogenesis.</td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>Impaired endothelial NO production and increased vasoconstrictor prostaglandins, glycated proteins, endothelium adhesion molecules and platelet and vascular growth factors enhance vasomotor tone and vascular permeability and limit growth and remodeling (Tooke, 1995).</td>
</tr>
<tr>
<td>Arterial stiffness</td>
<td>Increased central aortic pressure and left ventricular afterload and lowered central diastolic and coronary perfusion pressures, leading to subendocardial ischaemia and interstitial fibrosis (Vinereanu et al., 2003).</td>
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<tr>
<td>Autonomic</td>
<td>Decreased sympathetic/parasympathetic myocardial innervations neuropathy with impaired coronary resistance vessel vasodilator response and impaired ventricular diastolic filling (Monteagudo et al., 2000).</td>
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</tbody>
</table>
Figure 3.2: Potential contributors to the development of diabetic cardiomyopathy. TG indicates triglycerides; GLUTs, glucose transporters; PDK4, pyruvate dehydrogenase kinase 4; MCD, malonyl-coenzyme A decarboxylase; MCoA, malonyl-coenzyme A; ACoA, acetyl-coenzyme A; ACC, acetyl coenzyme A carboxylase; CPT1, carnitine palmitoyl-transferase 1; PDH, pyruvate dehydrogenase; CE, cardiac efficiency; PKC, protein kinase C; and AGE, glycation end products.
DIABETIC CARDIOMYOPATHY IN ANIMAL MODELS

Diabetic cardiomyopathy in experimental animal models of type 1 diabetes is characterized by phenotypic changes in the ventricular myocytes that occur in the presence or absence of coronary artery disease. This cardiomyopathy is well described in animal models with long-term type 1 diabetes and results in abnormal cardiomyocyte excitation-contraction (E-C) coupling [eg prolonged action potentials, slowed cytosolic Ca2+ effluxes and slowed myocytes shortening and re-lengthening (reviewed by Pierce and Russell, 1997 and Chatham et al., 1996)]. The cellular mechanisms that contribute to myocyte dysfunction involve depressed expression and function of SERCA and Na+/Ca2+ exchanger (Schaffer & Mozafferi, 1996). Regulation of E-C coupling is also impaired in diabetic hearts, such that β-adrenergic receptor signaling is depressed, which may result from changes in β-adrenergic receptor density or redistribution of β-adrenergic receptor subtypes (Dincer et al., 2001), or perhaps signaling downstream of the receptors (Tamada et al., 1998). Elevated protein kinase C (PKC) activity and changes in the expression of specific PKC isoforms are also found in type I diabetic hearts (Idris et al., 2001).

INDUCTION OF DIABETES

Experimental diabetes mellitus has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. However, to induce a notable form of diabetes, at least 90-95% of the pancreas has to be removed. Otherwise, the Langerhans islets in the remaining pancreas may undergo hypertrophy and secrete a sufficient amount of insulin for fulfilling the natural metabolic needs. The second method for creating diabetes in animals is injecting drugs such as Alloxan or Streptozotocin (Szkudelski, 2001). These materials inflate and ultimately degenerate the Langerhans islets beta cells. A less reliable method for creating diabetes is injection of the anterior hypophysis extract. The final symptoms of insulin deficiency are clearly seen in rats afflicted with diabetes chemically by Streptozotocin (Elias et al., 1994).
MECHANISM OF STREPTOZOTOCIN ACTION

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is synthesized by *Streptomyces achromogenes* and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively). STZ is a broad-spectrum antibiotic with oncolytic, oncogenic, and diabetogenic properties (Rossini et al., 1977). The diabetogenic action is mediated by selective destruction of pancreatic beta cells and has been widely utilized as a method for inducing diabetes mellitus in experimental animals and for treatment of malignant beta cell tumors and other neoplasm’s in humans. To produce diabetes, STZ is conventionally administered as a single injection. STZ is cleared from the bloodstream rapidly (serum half-life, 15 min); beta cell necrosis can be detected by electron microscopy within hours after STZ injection. Elevated blood glucose levels are demonstrable within 1-2 days, and dissolution and phagocytosis of necrotic cells are observed histologically after 3 days (Szkudelski, 2001). In rats and mice, the administration of a single subdiabetogenic dose produces only mild histologic alterations without evidence of significant hyperglycemia when compared with buffer-injected control animals.

The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w., but higher doses are also used (Szkudelski, 2001). STZ is also efficacious after intraperitoneal administration of a similar or higher dose. Streptozotocin enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA (Figure 3.3). DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, B cells undergo the destruction by necrosis. The injection of streptozotocin (STZ) in rats leads to the development of a clinical syndrome characterized by hyperglycemia, excessive osmotic diuresis and loss of weight, which is similar to human diabetes (Akbarzadeh et al., 2007).
MYOCARDIAL FUNCTIONS IN EXPERIMENTAL DIABETES

In animal models of diabetes, several functional and structural alterations of the heart or in cardiac muscle have been documented. Most studies have been performed in isolated perfused hearts and reveal depressed cardiac function (Severson, 2004; Buchanan et al., 2005). Fewer studies have reported normal function in vitro (Sidell et al., 2002; Wang et al., 2005).

Besides increased incidence of autonomic neuropathy and coronary artery disease, diabetes has been considered an independent risk for myocardial dysfunction (Hayat et al., 2004). It is well documented in experimental diabetes that both left ventricular diastolic and systolic dysfunction are attributed to diabetic cardiomyopathy, as usually seen under clinical condition. A deleterious effect of diabetes on cardiac performance has been well documented in isolated heart preparation, isolated cardiomyocytes and intact anesthetized animals (Severson, 2004). Besides myocardial dysfunction, it is well

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**Figure 3.3:** The mechanism of streptozocin (STZ)-induced toxic events in B cells of rat pancreas. MIT—mitochondria; XOD—xanthine oxidase.
known that experimental diabetes affects hemodynamic parameters such as heart rate and arterial pressure. It is well documented that insulin can prevent, or revert, a number of outcomes caused by experimental diabetes. In fact, insulin normalizes not only blood glucose levels, but most of the metabolic parameters of diabetic rats. Studies from isolated papillary muscle, isolated heart preparations, and conscious rats showed that insulin reversed, or prevented, contractile dysfunction in diabetic rats (Sasaki and Bunag, 1983).

Myocardial function was reported to be attenuated in conscious STZ-diabetic rats (Borges et al., 2006). In addition, the lower dosage of dobutamine uncovered a greater responsiveness of the myocardium of STZ-diabetic rats. Insulin preserved myocardial function and the integrity of the response to dobutamine of STZ-diabetic rats.

Myocardial dysfunction is an important feature that might be associated with a number of intrinsic alterations of cardiac myocytes (Ren and Bode, 2000). There are several studies in vivo (anesthetized animals) and in vitro (Langhendorff and isolated myocytes) showing an impairment on Ca++ homeostasis and Ca++signaling in diabetes (Choi et al., 2002). The literature reports studies of myocardial contractility in diabetes showing conspicuous diastolic dysfunction (Fein et al., 1980). Fein et al described substantial deleterious effects of diabetes in cardiac papillary muscles of rats. The most significant abnormalities involved delay of the relaxation process, slow relaxation ratio and delay in peak ratio of isometric and isotonic relaxation. Nevertheless, recent studies demonstrated reduced expression of sarcoplasmic reticulum calcium-ATPase and sodium-calcium exchanger (Choi et al., 2002). Decreased basal contractility in the STZ-diabetic rats, as demonstrated by means of low +dP/dtmax, was also observed (Borges et al., 2006). Reports in the literature have demonstrated myocardial dysfunction in spontaneously diabetic rats (Ren and Bode, 2000). It has been suggested that this myocardial derangement is typical of the cardiac myocyte of the diabetic rat, which shows significant reduction of cell shortening and attenuation of the velocities of cell shortening and relaxation, associated with low levels of cytosolic Ca++. The decrease in inward Ca++ current, which triggers the ryanodine Ca++ channel to release Ca++ (Choi et al., 2002) from the sarcoplasmic reticulum, could be also an important factor to explain the decrease of +dP/dtmax.
CARDIOVASCULAR AUTONOMIC NEUROPATHY IN DIABETES

CAN (cardiac autonomic neuropathy) may contribute to impaired diastolic function and is associated with an increased cardiovascular risk in diabetic patients. Diabetic autonomic neuropathy was associated with an impaired vasodilator response of coronary resistance vessels to increased sympathetic stimulation (Maser and Lenhard, 2005). Twenty one percent of patients with Type I diabetes without ischaemic heart disease have abnormal diastolic filling which is associated with the severity of CAN. Similarly ventricular filling abnormalities are most prominent in patients with autonomic neuropathy (Vinik and Ziegler, 2007). Mustonen et al (1992) have shown a correlation between myocardial sympathetic innervation derived from scintigraphy and the $E/A$ ratio (ratio of early to late peak mitral filling wave velocities) in Doppler echocardiography, providing evidence that an abnormal sympathetic innervation of the heart may contribute to a disturbance in LV filling. Sympathetic dysfunction has been related to both systolic and diastolic dysfunction in Type II diabetes (Annonu et al., 2001). An abnormal systolic blood pressure response to standing was correlated significantly with a reduced mitral $E/A$ ratio. Studies also reflect an association between parasympathetic and cardiac dysfunction as evidenced by the association between significantly lower mean heart rate variation during deep breathing and abnormal diastolic peak filling rate in diabetic patients (Uusitupa et al., 1988). The mitral $E/A$ ratio has been shown to be significantly reduced in patients with autonomic neuropathy and a significant correlation was observed between the $E/A$ ratio and autonomic neuropathy (Monteagudo et al., 2000).

AUTONOMIC CONTROL IN EXPERIMENTAL DIABETES

The STZ-diabetic rat develops the usual chronic microvascular complications (nephropathy, peripheral and autonomic neuropathy) as observed in diabetic patients. Studies on 5-day STZ-diabetic rats have shown depressed vagal tone, reduction of vagal effect and impaired tachycardic response to arterial pressure (AP) decreases (Maeda et al., 1995). Fifteen days after STZ injection impairment of the reflex bradycardia and tachycardia produced by vasopressor and vasodepressor agents, respectively was observed. Similar cardiovascular changes were described in this model and there is
evidence that some of these alterations are reversed by insulin therapy (Dall Ago et al., 1997). These findings, associated with the impairment of baroreflex sensitivity, an excellent gauge of autonomic function, suggested the early development of autonomic dysfunction in these animals. Since the autonomic nervous system modulates beat-to-beat fluctuations in heart rate (HR), methods to quantify HR and blood pressure variability have been evaluated as indicators of sympathetic and parasympathetic modulation of the cardiovascular system in humans and in experimental models. These methods seemed to detect early autonomic dysfunction at a time when other metabolic dysfunctional changes were not clearly observed.

BAROREFLEX SENSITIVITY

The evaluation of baroreflex sensitivity (BRS) is an established tool for the assessment of autonomic control of the cardiovascular system. Besides the well-acknowledged physiological role in the maintenance of circulatory homeostasis, evidence has been accumulated that changes in the characteristics of baroreflex function reflect alterations in autonomic control of the cardiovascular system (La Rovere et al., 1998). Arterial baroreceptors provide the central nervous system with a continuous stream of information on changes in blood pressure (which are sensed by the stretch receptors in the wall of the carotid sinuses and aortic arch), on the basis of which efferent autonomic neural activity is dynamically modulated. Activation of arterial baroreceptors by a rise in systemic arterial pressure leads to an increase of the discharge of vagal cardioinhibitory neurons and a decrease in the discharge of sympathetic neurons both to the heart and peripheral blood vessels. This result in bradycardia decreased cardiac contractility and decreased peripheral vascular resistance, and venous return (La Rovere et al., 2008). Conversely, a decrease in systemic arterial pressure causes the deactivation of baroreceptors with subsequent enhancement of sympathetic activity and vagal inhibition, leading to tachycardia and increase of cardiac contractility, vascular resistance, and venous return.

BAROREFLEX ASSESSMENT

In humans several techniques have been used to measure baroreflex gain. Quantification of BRS has been obtained by measuring the change in heart rate in response to changes
in blood pressure induced by injection of vasoactive drugs that have minimal effect on the sinus node. However, the need for intravenous cannulation and the use of a drug limits the applicability of these techniques. Noninvasive alternatives are mainly represented by the Valsalva maneuver, the neck chamber technique (which provides a selective manipulation of carotid baroreceptors), and the analysis of spontaneous variations of blood pressure and RR interval.

Among the pharmacological perturbations, vasoconstrictor drugs have been the most widely used in the clinical setting. Smyth et al (1969) first measured the bradycardia produced in humans by an intravenous bolus of a pressor drug. The use of angiotensin as a pressor agent was subsequently replaced by the use of phenylephrine, a pure α-adrenoreceptor agonist, devoid of direct effects on cardiac contractility and the central nervous system.

While vasoconstrictor drugs mainly explore the vagal component of the baroreceptor control of heart rate, the excitation of the sinus node that accompanies a reduction in arterial pressure caused by the administration of vasodilators is partly mediated through sympathetic mechanisms. Therefore these drugs have been used to obtain information on the sympathetic limb of heart rate control. The injection of 100–200 mcg of nitroglycerin determines an immediate and progressive fall in systolic arterial pressure of about 20 mmHg over the following 8–15 beats (Osculati, 1990). Baroreflex slopes obtained by vasodilators are lower than those obtained by increasing arterial pressure to a similar extent, suggesting that the two responses are not mirror images; yet a direct effect of the vasodilator drug on pacemaker cells cannot be excluded (Pickering et al., 1972).

Several investigators have studied the baroreflex function in hyperglycemic rats treated with streptozotocin (Chang and Lund, 1986; Maeda et al., 1995). The reflex tachycardic response elicited by arterial pressure (AP) reduction was found to be attenuated in short-term diabetes (Maeda et al., 1995), while the reflex bradycardia in response to an AP increase has been reported to be normal in short-term diabetes. In contrast, baroreflex-mediated bradycardia during increasing AP is impaired in alloxan-induced diabetic rabbits at the same time that reflex tachycardia to AP reduction is preserved (McDowell et al., 1994). Jackson and Carrier (1983) and Homma et al.,
(1993) have reported an enhancement in baroreflex function when vasopressor or depressor agents are exogenously administered and when the right cervical sympathetic or vagus nerve is electrically stimulated, respectively. Several lines of evidence indicate that changes in baroreflex function are probably due to peripheral dysfunction of the autonomic nervous system in diabetic patients and animals (Dall'Ago et al., 1997).

Time-dependent changes in HR control were observed in earlier studies. Early in the course of experimental diabetes there was an impairment of baroreflex control in STZ rats characterized by reduction of baroreflex-mediated tachycardia, while baroreflex-mediated bradycardia was still maintained (Maeda et al., 1995). Later (15 and 30 days after STZ), the baroreflex-mediated bradycardia was also lost in diabetic rats (Dall'Ago et al., 1997), and these changes persisted even 80 days after STZ injection (De Angelis, 1999). On the other hand, McDowell et al. (1994) observed maintenance of the response to the increase in blood pressure induced by infusion of a vasoconstricitor agent 2 weeks after STZ treatment, but their study was conducted on rabbits. Also in a different animal model, the spontaneously diabetic rat (Bio-Breeding), Krizsan-Agbas and Buñag (1991) demonstrated exacerbation of baroreflex-mediated tachycardia, while Eckberg et al. (1986) reported a normal tachycardia response to decreases of blood pressure in diabetic humans.

The impaired ability to perform adequate HR regulation during changes in arterial pressure has been attributed to some alterations in cardiac parasympathetic activity, although changes in the receptor function or in the central mediation of the baroreceptor reflex cannot be excluded (De Angelis et al., 2002). The parasympathetic dysfunction could be due to alterations in cardiac muscarinic receptors (Carrier & Aronstam, 1987). It was reported that the density of cardiac muscarinic receptors was unaltered in STZ-diabetic rats. However, these investigators did not measure the density of atrial muscarinic receptors separately. Carrier et al. (1984) demonstrated that there was no difference in muscarinic receptor density in ventricles from STZ-diabetic and age-matched control rats, but the density of muscarinic receptors was reduced in the right and left atria from diabetic rats (Carrier & Aronstam, 1987).
ELECTROCARDIOGRAM (ECG) IN RATS

Extensive research has lead to a growing appreciation that the heart is acutely sensitive to a broad array of toxicants via multiple routes of exposure. Adverse effects in the heart often manifest as a change in the electrocardiogram (Farraj et al., 2011). In toxicology, the ECG provides a collection of end points that may be used to assess both the quality and magnitude of cardiac toxicity. Increasingly over the last two decades, the cardiotoxicity of agents have been characterized using small rodent electrocardiography. Additionally, tremendous insight into possible mechanisms of action of known human cardiotoxicants has been gained. Thus, the incorporation of small rodent electrocardiographic assessments into toxicology studies may facilitate the screening of cardiotoxic potential and the elucidation of mechanisms of action. Figure 3.4 illustrates the human and rat/mouse hearts highlighting the propagation of the action potential wave and ECG.

The landmarks within the ECG represent an ordered sequence of electrical events with the PR interval reflecting conduction between the sinus node in the right atrium and the ventricular endocardium, the QRS complex representing total ventricular activation, with the remaining portions of the ECG (QT interval, ST segment and T waves) reflecting ventricular repolarization. Prolonged PR intervals may suggest AV block (block of conduction to ventricles) or proximal conduction system disease (Berne and Levy, 2001). QRS changes (e.g., prolongation or fractionation) may identify individuals with increased arrhythmia risk and have been attributed to bundle branch blocks or ventricular ectopic foci (abnormal pacemaker sites in ventricles, Berne and Levy, 2001). ST segment depression is characteristic of myocardial (ventricular) ischemia/hypoxia, and ST segment elevation is characteristic of myocardial injury (Berne and Levy, 2001). Ischemic episodes in humans are negative prognostic indicators that point to an increase in the probability of future cardiac events including myocardial infarction (Tzivoni et al., 1988). Abnormal T-wave morphology may indicate underlying pathology. The QT interval, when adjusted for heart rate (corrected QT; QTc), is used to assess abnormalities in ventricular repolarization (because Q waves are often not present in rat and mouse ECGs, the base of the R wave is used in rodent models as a surrogate for the Q). Spatiotemporal heterogeneity of repolarization (as indicated by abnormal T waves
and/or QT intervals) may trigger arrhythmias and thus has been used to identify patients at risk for cardiac death (Henneberger et al. 2005).

The pathophysiological changes associated with toxicant exposure or disease manifest similarly in the ECG of rodents and humans. For example, the pathological Q wave, a hallmark sign of myocardial necrosis in human myocardial infarction, has been demonstrated in multiple rat models of myocardial infarction and cardiomyopathy (Bestetti and Oliveira, 1990; Carll et al., 2010). Other ECG changes (including P-wave enlargement, prolonged PR interval, lengthening QRS, QRS axis deviation, and T-wave changes), when compared with concomitant assessments of histopathology, have a high predictive value for many forms of heart disease in the rat (Bestetti and Oliveira, 1990). In addition, the QT interval is positively correlated with left ventricular mass in
hypertensive rats with reversal of both after antihypertensive therapy, thus demonstrating
that the QT interval reflects the phenotypic changes of a mechanically stressed heart
(Baillard et al., 2000; Barr et al., 1994).

The QT interval is dependent on the heart rate in an obvious way (the faster the heart
rate the shorter the QT interval) and may be adjusted to improve the detection of patients
at increased risk of ventricular arrhythmia. The corrected QT interval (QTc) is
calculated by dividing the QT interval by the square root of the preceding R - R interval
(Bazett, 1920). Normal = 0.42 s. Prolonged QTc indicates cardiomyopathy.

Electrocardiographic changes in raw and corrected QT intervals and R wave amplitudes
are early indicators of evolving cardiovascular disease and increased cardiovascular risk
in diabetes. Prolonged QT and QTc intervals are considered reliable predictors of heart
disease and fatal ventricular arrhythmias (Henneberger et al. 2005). A positive linear
relationship exists between QTc interval prolongation and diabetic cardiac autonomic
neuropathy (CAN) severity in diabetic population (Cardoso et al., 2003). Heart rate
variability (HRV), one indicator of CAN, decreases with diabetes which indicates
increased mortality risk. QT and QTc interval abnormalities reflect changes in cardiac
architecture. A positive correlation between QT or QTc interval prolongation and left
ventricular (LV) mass has been reported (Christensen et al., 2000). LV hypertrophy
presents as exaggerated R wave amplitudes on ECG recordings. Elevated R wave
amplitudes are an independent risk factor for cardiovascular events. LV hypertrophy and
QT interval alterations coupled with decreased cardiac function are commonly observed
with diabetes related cardiovascular disease. Abnormalities of the T wave and ST
segment are also reported in diabetic ketoacidosis (Klien et al., 2005).

ROLE OF LIPIDS IN DIABETIC COMPLICATIONS

Diabetes mellitus is associated with a large number of lipid abnormalities. Emerging
evidence confirms the pivotal role of hyperlipemia, mainly elevated blood cholesterol,
particularly LDL cholesterol and VLDL cholesterol in the development of atherosclerosis-
related disease (Pyorala et al., 1987). Significant abnormalities in lipid metabolism and
lipoproteins in diabetes are evident which in turn depend on the extent of insulin
deficiency, insulin resistance, obesity, diet and the presence of concomitant primary and
other secondary causes of hyperlipemia (Andallu et al., 2009). In diabetic hyperlipemia, a series of bizarre lipoproteins and other lipids appear and interaction of this with oxidative stress and free radicals leads to enhanced lipid peroxidation in plasma, tissues and membranes, causing extensive tissue damage. It is well known that lipid peroxidation provides a continuous supply of free radicals that play an important role in etiopathogenesis of diabetes and its complications (Kannel, 1985). Control of hyperlipidemia is a prerequisite for the prevention of diabetic microangiopathy (retinopathy, nephropathy and neuropathy) and macroangiopathy (ischemic heart disease), cerebral vascular disease (CVD) and arteriosclerosis obliterans in diabetes. Various therapeutic methods used in diabetes treatment available today achieve transiently regulated euglycemia but fail to prevent lipid and lipoprotein alterations, ultimately, exposing the diabetic humans and animals to cardiovascular complications. (Griesmacher et al., 1995)

The changes in adipose tissue lipolysis or intrahepatic mechanisms involving other changes in fractional esterification of fatty acids are responsible for the increase in triacylglycerol secretion rate. In vitro studies have shown a decrease in fractional catabolic rate for LDL from type 2 DM subjects and also evidence suggests that in vivo nonenzymatic glycosylation of LDL may result in decreased LDL clearance (Howard, 1987). LDL cholesterol concentrations are strongly and positively related to atherosclerotic complications (Castelli et al., 1986). Apart from this, glycation induces compositional and structural changes in LDL. Glycated LDL interacts with platelets leading to the development of vascular complications in diabetes by altering platelet aggregation, platelet nitric oxide production, intracellular Ca$^{2+}$ concentration, activities of Na$^{+}$ - K$^{+}$ and Ca$^{2+}$ ATPases (Ferretti et al., 2002). Increased glycation of apolipoproteins may play a role in the accelerated development of atherosclerosis in diabetes and altered activity of glycated LDL receptor contributes for hyperlipidemia. In addition, glycation of lipoproteins may also generate free radicals increasing oxidative damage to the lipoproteins themselves. Glycoxidation and browning of sequestered lipoproteins may further enhance their atherogenicity. The more severely modified (glycoxidized) lipoproteins in vessel walls may behave as more potent antigens than less modified particles found in the plasma stimulating the in situ formation of atherogenic immune complexes (Lyons, 1992).
In addition to glucose, metabolic intermediates, such as triose phosphates, glyoxal and methylglyoxal, are recognized as important precursors of AGEs, and changes in the concentrations of these compounds, both inside and outside the cell, will affect the rate of AGE formation. There is also growing evidence from several clinical trials that dyslipidaemia, including hypertriglyceridaemia, is a significant and independent risk factor for diabetic complications (Januszewski et al., 2003). The chemical modification of proteins by lipids may be an underlying pathogenic mechanism linking dyslipidaemia to diabetic complications. This proposal is based on the fact that lipid peroxidation produces several dicarbonyl intermediates that are identical to those formed during glycoxidation reactions, including glyoxal and methylglyoxal [Fuller et al 2001], which are precursors of the major AGEs, including CML \( [\text{N}^\varepsilon-(\text{carboxymethyl})\text{lysine}] \), CEL \( [\text{N}^\varepsilon-(\text{carboxyethyl})\text{lysine}] \), imidazolone derivatives of arginine, and argpyrimidine. Recent work in animal models suggests that these dicarbonyl precursors may, in fact, be derived primarily from lipid peroxidation reactions, and that the major ‘AGEs’ in tissues may, in fact, be ALEs, derived from lipids. Increased lipid peroxidation and accelerated ALE formation, possibly catalyzed by hyperglycemia and oxidative stress, may be the mechanistic link between dyslipidaemia and diabetic complications.

**OXIDATIVE STRESS IN DIABETIC COMPLICATIONS**

Diabetes and its cardiovascular complications are related to multiple pathogenic factors, including hyperglycemia, hyperlipidemia, and inflammatory response. However, the pivotal mediator for the pathogenesis of diabetes and its cardiovascular complications is oxidative stress, directly or indirectly derived from the multiple factors mentioned above (Cai and James Kang, 2001). Oxidative stress is defined as the imbalance between the production of reactive oxygen and nitrogen species (ROS and RNS) and antioxidant capacity (Wold et al., 2005). Diabetes impairs cardiac antioxidant capacity, showing decreases in enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (vitamin C, E, or A) antioxidant defenses, as well as total radical-trapping antioxidant capacity in the heart. In addition to impaired defenses, diabetes also causes ROS and RNS overproduction in the hearts of diabetic animals and patients. Increased ROS production is implicated in the development of cellular hypertrophy and remodeling, at least in part through activation of redox sensitive protein kinases such as the mitogen-activated protein kinase (MAPK) superfamily. The transition from compensated pressure-
overload LVH to heart failure is associated with increased oxidative stress, which may promote myocyte apoptosis and necrosis. Several key proteins involved in excitation-contraction coupling, such as sarcolemmal ion channels and exchangers and sarcoplasmic reticulum calcium release channels, can undergo redox-sensitive alterations in activity, which contributes to myocardial contractile dysfunction. ROS also has indirect effects resulting from increased inactivation of NO and consequent generation of peroxynitrite, eg coronary vascular endothelial dysfunction and peroxynitrite-induced inhibition of myocardial respiration (Boudina and Dale Abel, 2007).

Several mechanisms have been proposed to explain how all of the pathologies involved in the progression of diabetic cardiomyopathy can result from hyperglycemia. Four main hypotheses have been presented to describe how hyperglycemia can cause all of these diabetic complications (Brownlee, 2001): increased polyol pathway flux, increased advanced glycation end-product (AGE) formation, increased protein kinase C isoform expression, and increased hexosamine pathway flux (Figure 3.5). All of these pathways, as well as several others, lead to hyperglycemia and increased reactive oxygen species (ROS) formation, causing diabetic cardiomyopathy.

Figure 3.5: Schematic diagram showing the possible contributing factors to oxidative stress en route to the onset of diabetic cardiomyopathy in both type 1 and type 2 diabetes.
The importance of AGEs in the development of diabetic complications is seen in the observation that two structurally similar AGE inhibitors partially prevented diabetic complications in the retina, kidney and nervous system (Soudis-Liparota et al., 1991; Nakamura, 1997; Hammes, 1991). One of the mechanisms how AGE precursors target cells is through the binding of AGE receptors to endothelial cells, mesangial cells and macrophages, inducing receptor-mediated production of ROS. This receptor ligation increases the production of the transcription factor NF-κB, also causing increased oxidative stress.

**Alterations in stress signaling pathways**

Hyperglycemia in diabetes causes changes in membrane function and metabolic and biochemical alterations within days, changes in contractile function within weeks, and morphological changes and heart dysfunction within months (Chatham, 1996). A significant increase in oxidative damage via lipid peroxidation was observed in the hearts of diabetic rats (Kakkar et al., 1995). Production of hydroxyl radicals was also detected in diabetic rats induced by streptozotocin (Ohuwa et al., 1995). In the heart, hydroxyl radical production and elevated blood glucose concentration were directly correlated with the amount of STZ injected into rats, up to 60 mg/kg body weight (Ohuwa et al., 1995). With the use of fluorescent probes, myocytes isolated from STZ-induced diabetic mice were used to detect hydrogen peroxide and hydroxyl radicals, and increased ROS was observed compared with control mice (Kajstura et al., 2001). Oxidative damage caused by ROS has been shown to lead to multiple complications of diabetes (Ustinova et al., 2000; Rosen et al., 2001). Blocking ROS and superoxide formation, however, has been shown to prevent hyperglycemia-induced organ damage in diabetes (Nishikawa et al., 2000).

Cell death is an important determinant of cardiac remodeling because it causes a loss of contractile units, compensatory hypertrophy of myocardial cells and reparative fibrosis (Kang, 2001). Apoptotic cell death associated with increased oxidative stress in multiple organ systems of diabetes mellitus has been well documented (Cai et al., 2000; Srinivasan et al., 2000). Recent *in vivo* experiments have demonstrated the induction of myocardial cell apoptosis in experimental diabetic rats (Fiordaliso, 2000), mice
(Kajstura, 2001) and diabetic patients (Frustaci, 2000). Heart specimens from diabetic patients (both hypertensive and non-hypertensive) showed an increase in myocyte, endothelial and fibroblast apoptosis (Frustaci, 2000). The increased cell death was associated with an increase in ROS formation (Frustaci, 2000; Kajstura, 2001). However, the precise mechanism(s) by which ROS accumulation leads to compromised heart function and the effect of antioxidant therapy in diabetic subjects is largely unknown. Therefore, it is important to study the signaling pathways and molecular mechanisms by which hyperglycemia-induced (or, presumably, STZ-induced) oxidative stress leads to cell death and myocardial pathogenesis.

**Role of antioxidants in diabetic cardiomyopathy**

Mitochondrial damage is related to ROS formation and plays an important role in the development of diabetic cardiomyopathy (Tomita et al., 1996; Kucharska et al., 2000). Coenzyme Q (CoQ) is an important component in mitochondrial energy metabolism and is also a potent endogenous antioxidant *in vivo*. In heart mitochondrial preparations of diabetic rats, the concentration of α-tocopherol was increased; however, the concentration of both CoQ-9 and CoQ-10 was decreased (Kucharska et al., 2000). It has been shown that the reduction in coenzyme levels from diabetic animals is attenuated with the supplementation of insulin-like growth factor I (IGF-1, Norby et al., 2002). It is important to note that contractile function of the heart requires a high metabolic demand, and the mitochondrial respiratory chain is the primary energy-releasing system in the myocyte. Through the respiratory chain, a series of oxidation-reduction reactions continually take place in the myocyte. Therefore, an efficient antioxidant system, including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione (GSH) and α-tocopherol are critical to effective functioning of the myocardium. However, in experimental animal models, the heart levels of these antioxidants are much lower than in other organ systems, even in non-diabetic normals (Chen, 1994). In addition, hyperglycemia can impair and decrease the amount of antioxidants within the heart of a diabetic animal (Elangovan et al., 2000) making it more vulnerable to ROS-induced damage. The increase in ROS serves to decrease the antioxidant capacity of the diabetic myocardium, contributing significantly to oxidative
stress and resultant myocardial damage. This damage causes cardiac morphological and functional abnormalities. Epstein and colleagues (Ye et al., 2003; Ye et al., 2004) showed that type 1 diabetic cardiomyopathy could be prevented when the antioxidants metallothionein (MT) and catalase were overexpressed specifically in the heart. They also showed that ROS production was enhanced in genetically diabetic mice (OVE26), which could be prevented by genetically crossing the diabetic mice with those overexpressing the MT or catalase genes.

OXIDATIVE STRESS AND DIABETIC NEUROPATHY

In the diabetic state, unchecked superoxide accumulation and resultant increases in polyol pathway activity, AGE accumulation, PKC activity, and hexosamine flux trigger a feed forward system of progressive cellular dysfunction (Figure 3.6). The diabetic state produces impaired neurotropism, axonal transport and gene expression through at least four major pathways (Feldman, 2003).

1. Excess glucose is diverted away from glycolysis by the polyol pathway that depletes NADPH and cellular antioxidant capacity.

2. Glucose also may become oxidized and form AGEs that alter extracellular matrix, activate receptors that produce ROS intermediates, and alter intracellular protein function.

3. PKC becomes activated either directly by glycolytic intermediates or indirectly as shown as a second messenger for stress hormones, leading to increased vascular disease, inflammation, and oxidative stress.

4. Partial glycolysis causes accumulation of glycolytic intermediates and leads to escape of fructose-6-phosphate along the hexosamine pathway that increases vascular disease and further ROS generation. These mechanisms are ultimately linked to superoxide production through increased glucose respiration that produces superoxide in the mitochondria and also activates the superoxide-producing NADH oxidase.
In nerve, this confluence of metabolic and vascular disturbances leads to impaired neural function and loss of neurotrophic support, and long term, can mediate apoptosis of neurons and Schwann cells, the glial cells of the peripheral nervous system (Feldman, 2003; Schmeichel, 2003). Decreases in nerve growth factor (NGF), neurotrophin-3 (NT-3), ciliary neurotrophic factor, and IGF-I in nerves from animals with experimental diabetes are well documented and correlate with the presence of neuropathy (Apfel, 1999; Tomlinson, 1997).

The elegant work of Calcutt and colleagues reports a decrease in desert hedgehog expression in nerves from young adult rats with streptozotocin-induced diabetes (Calcutt, 2003). Hedgehog proteins (sonic, desert, and indian) are essential for normal nervous system development (Parmantier, 1999). Desert hedgehog is found exclusively in the peripheral nervous system in Schwann cells and is important in peripheral nerve patterning (Parmantier, 1999). After 10 weeks of experimental diabetes, Calcutt et al. observed a decrease in desert hedgehog gene expression. This decrease correlates with
several well established physiological and biochemical markers of experimental diabetes, including slowed motor and sensory nerve conduction velocities, decreased nerve blood flow, decreased pain threshold in response to heat and/or formalin, and decreased NGF and neuropeptide levels. Thrice weekly injections of sonic hedgehog linked to an IgG fusion protein, beginning after 5 weeks of experimental diabetes and continuing for an additional 5 weeks, restored motor and sensory nerve conduction velocities and both NGF and neuropeptide levels. There was no therapeutic effect on nerve blood flow or pain threshold levels. Morphometric analyses of sciatic nerves revealed that diabetic animals had a decrease in medium sized myelinated fibers, which was restored by sonic hedgehog treatment.

While purely speculative, it is likely that restoration of hedgehog activity provided much needed neurotrophic support both directly, by activating hedgehog downstream pathways and indirectly, by restoring NGF levels. Hyperglycemia induced decreases in neurotrophic factors are well documented, with neurotrophic replacement frequently restoring one or more impaired nerve parameters to normal. Administration of NGF restores neuropeptide levels and sensory amplitudes in experimental diabetes (Goss, 2002; Tomlinson, 1997); in parallel, NT-3 normalizes nerve conduction slowing (Mizisin et al., 1999; Pradat, 2001) and IGF-I administration blocks the development of neuropathy and reverses impaired nerve regeneration (Schmidt, 1999). When oxidative stress is induced in nerves of nondiabetic animals by administering pro-oxidants, decreases in NGF and NT-3 are observed similar to those reported in animals with experimental diabetes (Hounsom, 2001). Antioxidant therapy in experimental diabetic neuropathy blocks the observed decreases in nerve NGF and restores nerve function (Obrosova, 2001). Antioxidant therapy also restores normal blood flow and nerve conduction velocities in experimental diabetes (Tomlinson, 1998; Greene et al., 1999). Interestingly, neurotrophic factors may also serve as antioxidants and this function may contribute to their role as possible therapeutic entities in diabetic neuropathy (Pan et al., 1997; Park, 1998).

**ROLE OF INFLAMMATORY CYTOKINES**

Proinflammatory cytokines specially, interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), are capable of modulating cardiovascular function (Dinh et al., 2009). They also exhibit pleiotrophic effects on homeostasis of glia and neurons in central,
peripheral, and autonomic nervous system. These cytokines are produced locally by resident and infiltrating macrophages, lymphocytes, mast cells, fibroblasts, and sensory neurons (Skundric and Lisak, 2003). Metabolic changes induced by hyperglycemia lead to dysregulation of cytokine control increasing their levels by an oxidative mechanism (Esposito et al., 2002). Tumour necrosis factor (TNFα) was first described in 1975 and termed cachectin. In 1990 Levine and associates observed that mean (SEM) serum concentrations of TNFα were higher in CHF patients than in healthy subjects. They also demonstrated that those patients with high concentrations of TNFα were more often suffering from cardiac cachexia. TNFα exerts its effects via TNFα receptors (TNFR), which are expressed by almost all nucleated cells. Two TNFRs have so far been identified. TNFR-1 is more abundantly expressed and appears to be the main signaling receptor. The majority of deleterious effects caused by TNFα seem to be mediated via this receptor, whereas TNFR-2 appears to have a more protective role in the heart. Previous studies have identified both types of receptors in non-failing and failing human myocardium. After translation, both receptors, like TNFα itself, are inserted into the cell membrane of the respective cell.

TNFα has been implicated in the development of left ventricular dysfunction, left ventricular remodelling, increased cardiac myocyte apoptosis, the development of anorexia and cachexia, reduced skeletal muscle blood flow and endothelial dysfunction, severity of insulin resistance, activation of the inducible form of nitric oxide synthase (iNOS), β receptor uncoupling from adenylate cyclase, and other effects (Lechleitner, 2000).

**Inflammatory cytokines in diabetic cardiomyopathy**

There is increasing evidence that inflammation is involved in the pathophysiology of heart failure and diabetes (Dinh, 2009). Most recent studies have linked insulin-resistance with TNF-α and IL6 and those studies have shown that a measure of proinflammation is predictive for type 2 diabetes. Furthermore, increased circulating concentrations of IL-6 and TNF-α were found in Type 2 DM and impaired glucose tolerance (Muller et al., 2002; Pickup et al., 2000). Left ventricular diastolic dysfunction (LVDD) is considered a precursor of systolic heart failure and diabetic cardiomyopathy (Piccini et al., 2004) and is common in the community and especially in diabetic patients.
(Futh et al., 2009). Increased plasma levels TNF-α and IL-6 seem to be correlated with impaired LVDD and more advanced left ventricular diastolic dysfunction. These data provide clinically important information on systemic immune abnormalities in subjects with LVDD. The Framingham Heart Study was the first to demonstrate an increased risk of heart failure in patients with diabetes (Kannel et al., 1976). Since then, additional studies, including SOLVD (Shindler et al., 1996) and HOPE (Arnold et al., 2003), have identified diabetes as a major risk factor for the development of heart failure. It has been demonstrated that IL-6 shows cardiodepressive properties (Finkel et al., 1992). In patients with systolic heart failure, IL-6 and TNF-α are associated with functional NYHA class (Torre-Amione et al., 1996). Furthermore, IL-6 and TNF-α have been shown to be independent predictors of mortality in heart failure (Deswal et al., 2001).

Proinflammatory cytokines are capable of modulating cardiovascular function by various mechanisms. It is now known that virtually every nucleated cell type in the myocardium, including the cardiac myocyte, is able to secrete proinflammatory cytokines in response to various myocardial damage or stressors. The expression of these cytokines can occur in absence of systemic immune activation. They partly act in a negative inotropic manner and cause changes in turnover of the extracellular matrix resulting in myocardial fibrosis. The proinflammatory cytokine TNF-α induces cardiodepressive effects and causes apoptosis (Ing et al., 1999). The development of progressive cardiomyocyte apoptosis plays a critical role on the left ventricular geometry and the adverse cardiac remodeling that occurs in the setting of sustained inflammation.

**Inflammatory cytokines in diabetic neuropathy**

Cytokines are involved in the pathogenesis of nerve damage as well as repair. For example, TNF-α injected into nerve induces inflammatory demyelination and wallerian degeneration, whereas IL-1 production promotes phagocytosis by scavenger macrophages and the synthesis of neurotrophic factors nerve growth factor (NGF) (Carlson et al., 1996). Proinflammatory cytokines are instrumental to the course of inflammatory demyelinating neuropathies. They increase vascular permeability of the blood-nerve barrier, which favors transmigration of leukocytes into nerve. They induce activation and proliferation of lymphocytes and macrophages and may have a direct myelinotoxic activity. In addition, downregulation of the immunosuppressive cytokine TGF-β1 may favor the nerve inflammatory reactions (Creange et al., 1997).
Mechanisms leading to nerve degeneration and regeneration are essential in the pathogenesis of diabetic neuropathy. Compared to normal, the ability of diabetic peripheral nerve to regenerate is significantly diminished (Cameron and Cotter, 1997). In a comparative analysis, Pierson et al. (2002) found that regenerative potentials in type I are affected to a greater extent than in type II diabetic neuropathy. The regulatory roles of cytokines in nerve degeneration and regeneration may potentially be utilized for the prevention and/or therapy of diabetic neuropathy.

The hallmarks of glucose metabolism through the polyol pathway are activation of the enzyme aldose reductase (AR) and accumulation of sorbitol. Hyperglycemia leads to accelerated phosphoinositide turnover, activation of phospholipase D and changes of DAG levels (Sugimoto et al., 2000). Although hyperglycemia in peripheral nerve leads to overall impaired phosphoinositide turnover, DAG accumulation and total protein kinase C (PKC) expression (Eichberg, 2002), there is evidence to suggest that vascular PKC-β isoforms are increased in diabetic nerve. Activated phospholipase D and DAG induce changes in the activation of PKC and expression of its isoforms in peripheral nerve. Changes in PKC activation and/or PKC isoform expression can modulate the activity of Na+, K+-ATPase. The Na+,K+-ATPase pump actively regulates intracellular concentrations of these two cations important for the function of cellular enzymes and transcription factors, such as activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB). Transcription factors AP-1 and NF-κB are involved in the initiation of the transcription of numerous genes, including proinflammatory and neuropoietic cytokines (IL-1, IL-6, TNF-α). In SCs, stimulation of PKC through extracellular signal regulated kinase (ERK) activation regulates the levels of LIFmRNA (Nagamoto-Combs et al., 1999). Neuropoietic cytokines have pleiotrophic effects on glia and neurons, either directly or indirectly through regulation of neurotrophic factors (Figure 3.7).

ROLE OF ENDOTHELIN 1

Since the discovery of endothelin-1(ET-1) as the most potent endothelial-derived vasoconstrictor/mitogenic peptide, considerable evidence has implicated this peptide in various cardiovascular disease states, including diabetes mellitus (Hopfner & Gopalakrishnan, 1999). Plasma and tissue concentrations of endothelin-1 as well as responses to the peptide are changed in various forms of the disease in humans and
animals. Endothelin activity is also altered in atherosclerotic and ischaemic disease, nephropathy, retinopathy, erectile dysfunction and neuropathy, many of the well-known complications of diabetes. Striking new evidence shows that antagonists of the endothelin system might beneficially affect and potentially overcome some of these complications.

**Figure 3.7:** Schematic diagram of major aberrant metabolic pathways induced by hyperglycemia. The proposed role of proinflammatory cytokines in relation to metabolic end products, activated enzymes, transcription factors, neuropoietic cytokines, and molecules involved in Schwann cell-axonal communication.
The cardiovascular Endothelin system

The endothelin (ET) family consists of three 21-amino acid peptides designated ET-1, ET-2 and ET-3 (Yanagisawa et al., 1998). The biological actions of the ETs are primarily mediated by two distinct, G-protein-coupled receptor subtypes designated ETA and ETB (Haynes & Webb, 1998). Endothelin-1 released by vascular endothelial cells exerts an autocrine influence by promoting vasodilatation, subsequent to activation of ETB receptors located on endothelial cells. It also exerts a paracrine effect on adjacent vascular smooth muscle cells (VSMC) in evoking vasoconstrictor and mitogenic actions by activation of both ETA and ETB receptors. The primary target of ET-1 is the vasculature where it evokes transient vasodilatation mediated by endothelial ETB receptors, followed by slow-onset and sustained contraction mediated by ETA and ETB receptors located on VSMC. The functional response to ET-1 varies throughout different tissues and vascular beds due to differences in distribution and expression of these two receptor subtypes.

Key metabolic variables that are changed in diabetes mellitus such as plasma insulin, glucose and lipids are well known to directly regulate the release of ET-1 from endothelial cells as well as modulate receptor expression and responses to the peptide. (Vane et al., 1990). Furthermore, prolonged and excessive exposure of both endothelial cells and VSMC to the metabolic dysregulation of diabetes mellitus could promote atherosclerotic and other morphological changes that indirectly affect the release and action of ET-1 at various target sites. Accordingly, alterations in ET-1 release and action have been consistently shown in both human diabetes mellitus and animal models of the disease. Morbidity associated with the diabetic state primarily results from pathological changes at the vascular level that logically fit with many of the known actions of ET-1.

Production and action of Endothelin 1 in diabetes

The status of ET-1 plasma concentrations in Type I (insulin-dependent) diabetes mellitus is controversial. The streptozotocin (STZ) diabetic rat, a widely used model of insulin deficiency characteristic of Type I diabetes, has been shown to have increased plasma ET-1 after 8 weeks (Makino & Kamata, 1998) and attenuated concentrations before 5 weeks (Hopfner et al., 1999) of exposure to diabetes. Accordingly, the duration of diabetes appears to determine the direction of changes in plasma ET-1 in this animal model (Hopfner et al., 1999). Caution is, however, warranted when interpreting plasma ET-1 concentrations in this model too soon after STZ treatment because actions of STZ
itself cannot be ruled out. In patients with Type I diabetes, both increased (Takahashi et al., 1990) and decreased (Smulders et al., 1994) plasma ET-1 has been reported and in patients with Type II (non-insulin-dependent) diabetes mellitus plasma ET-1 has been reported to be both increased (Donatelli et al., 1994) and unchanged (Guvener et al., 1997). Species differences as well as the concentration and duration of incubation of glucose used by these studies probably account for these discrepancies. Studies examining the effect of high glucose on receptors and responses to ET-1 have also yielded somewhat equivocal results. A summary of the effects of high glucose concentration on ET-1 release and action is shown in Figure 3.8.

ET receptor antagonists have been shown to be effective in several animal models, and initial clinical studies indicate that they also improve vascular function in patients with cardiovascular disease. The ETB receptor becomes of functional greater importance in several disorders like hypertension, atherosclerosis and insulin resistance (Ahlborg et al., 2007). This suggests that dual ETA/ETB receptor blockade may be superior to selective ETA receptor blockade in certain conditions by inducing vasodilatation, improving endothelial function and insulin sensitivity in humans, however further studies with head to head comparisons in various cardiovascular disorders are warranted.

Figure 3.8: Mechanism and consequences of ET alteration in diabetes. A schematic outline of various hyperglycemia-induced pathways leading to increases in ET levels (upper half of figure). Some of the major effects of increased ET levels are also presented (shown in italics; lower half of the figure).
ET-1 in Diabetic cardiomyopathy

ETs play important roles in several cardiovascular diseases, including diabetic cardiomyopathy, by modulating functional properties of cardiomyocytes and the microvasculature (Chen et al., 2000). In the heart, both cardiomyocytes and endothelial cells produce ET-1. Cardiac myocytes have high ET-1 binding affinity sites (Fukuchi & Giaid, 1998). ET-1 produces pronounced positive inotropic and chronotropic effects on the heart (Hu et al., 1988; Ishikawa et al., 1988). A duration dependent alteration of chronotropic and inotropic responses of ET-1 was demonstrated in the isolated atria of the diabetic rat (Lieu and Reid, 1994). Short-term hyperglycemia increased the expression of prepro ET-1 in the heart of STZ-diabetic rats (Erbas et al., 2000). Chen et al (2000) demonstrated a significant up-regulation of ET-1 and ETA and ETB receptor mRNA expression, as well as increased ET immunoreactivity and ET receptor density in hearts of 6-month diabetic rats (Chen et al., 2000). These changes were associated with focal apoptosis of cardiomyocytes, scarring of the myocardium, and increased fibronectin and collagen a1(IV) mRNA expression. Furthermore, such diabetes-induced abnormalities were completely prevented by bosentan (Chen et al., 2000). In humans, high plasma level of ET-1 is thought to play an important role in the pathogenesis of diastolic dysfunction in diabetic patients with cardiac autonomic neuropathy (Erbas et al., 2000). It has been further demonstrated that reperfusion following cardioplegia during coronary artery bypass grafting procedure can trigger the release of ET-1 in diabetic patients (Sharma et al., 1999), which may further contribute to significant cardiovascular demise in diabetic patients. Some of the effects of ET-1 on the heart and vasculature could be partially mediated via activation of sodium hydrogen exchanger 1 (NHE-1), the major proton pump mechanism in the heart, through the IP3-DAG pathway (Karmazyn et al., 1999). It has been demonstrated that both ET-1 and NHE-1 play important roles in the pathogenesis of diabetic heart disease. NHE-1 may act as the downstream mediator in the development of ET-mediated functional and structural changes in diabetic myocardium (Hileeto et al., 2001). PKC activation may be one of the pathways leading to ET up-regulation in the heart in diabetes (Ishii et al., 1996). However, several other factors may also be involved in the up-regulation of ET-1 expression in diabetes. ET-1 interacts with other potent vasoactive substances, such as
nitric oxide (NO) and vascular endothelial growth factor (VEGF, Hileeto et al., 2001). Increased VEGF in diabetes may also lead to increased ET-1 expression (Chen et al., 2000). On the other hand, nonenzymatic glycation and oxidative stress reduces NO production in diabetes, which in turn increases ET-1 expression (Nadler and Winer, 1996). It has been demonstrated that diabetes-induced reduction in NOS expression and NO activity in the heart may be corrected by treatment with bosentan (Mukherjee et al., 2001).

STATINS

Statins (or HMG-CoA reductase inhibitors) are a class of drug used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular diseases, and statins are therefore used in the prevention of these diseases. Statins are generally accepted as effective in decreasing mortality in people with preexisting cardiovascular disease. They are also currently advocated for use in patients at high risk of developing heart disease, but the evidence for this is questioned. A 2010 meta-analysis of studies found no benefit in terms of all-cause mortality when statins were used as a high-risk primary prevention intervention (Ray et al., 2010).

Statins play an important role in decreasing neointimal vascular occlusion and improving blood flow in the systemic arterial circulation (Dickinson et al., 2007). Treatment with statins, produce improvements in cardiovascular outcomes that are incompletely explained by reductions in cholesterol. Statins exert direct anti-inflammatory and antiproliferative effects on the components of vascular wall (Horwich, 2004). Although statins have been found to be effective in lowering serum low-density lipid levels by as much as 21% to 43%, they have been found to cause many adverse side effects. Statins are basically enzyme inhibitors, so it is likely that they may be inhibiting other critical enzymes in the body that have not been investigated so far, causing serious adverse side effects. Statins are ingested on a long-term basis to produce and maintain the desirable effect, therefore there may be a risk of chronic toxic effects including carcinogenic, teratogenic, and mutagenic, over a lifetime of use (Gotto, 2006).
Statin: Mechanism of action

Statins act by competitively inhibiting HMG-CoA reductase, the first committed enzyme of the HMG-CoA reductase pathway. Because statins are similar to HMG-CoA on a molecular level they take the place of HMG-CoA in the enzyme and reduce the rate by which it is able to produce mevalonate, the next molecule in the cascade that eventually produces cholesterol, as well as a number of other compounds. This ultimately reduces cholesterol via several mechanisms (Figure 3.9). By inhibiting HMG-CoA reductase, statins block the pathway for synthesizing cholesterol in the liver. This is significant because most circulating cholesterol comes from internal manufacture rather than the diet. When the liver can no longer produce cholesterol, levels of cholesterol in the blood will fall. Cholesterol synthesis appears to occur mostly at night (Miettinen, 1982), so statins with short half-lives are usually taken at night to maximize their effect. Studies have shown greater LDL and total cholesterol reductions in the short-acting simvastatin taken at night rather than the morning (Wallace et al., 2003), but have shown no difference in the long-acting Atorvastatin (Cilla et al., 1996).

Effects of statin

Statins exhibit action beyond lipid-lowering activity in the prevention of atherosclerosis. The ASTEROID trial showed direct ultrasound evidence of atheroma regression during statin therapy (Nissen et al., 2006). Researchers hypothesize that statins prevent cardiovascular disease via four proposed mechanisms (Furberg, 1999):

1. Improve endothelial function
2. Modulate inflammatory responses
3. Maintain plaque stability
4. Prevent thrombus formation

Statins may even benefit those without high cholesterol. In 2008 the JUPITER study showed fewer stroke, heart attacks, and surgeries even for patients who had no history of high cholesterol or heart disease, but only elevated C-reactive protein levels. There were also 20% fewer deaths (mainly from reduction in cancer deaths) though deaths from cardiovascular causes were not reduced (Ridker et al., 2008).
Adverse effects of statins

Statins have been reported to cause adverse effects like muscle toxicity, including myopathy and rhabdomyolysis, and effects on liver enzymes (Silva et al., 2006). It decreases ubiquinone (coenzyme Q10), an antioxidant molecule leading to a state of pro-oxidant stress in heart failure. Some patients on statin therapy report myalgias, muscle cramps or less frequently, gastrointestinal or other symptoms. Liver enzyme
derangements may also occur, typically in about 0.5%, are also seen at similar rates with placebo use and repeated enzyme testing, and generally return to normal either without discontinuance over time or after briefly discontinuing the drug (Golomb and Evans, 2008). A Danish case-control study published in 2002 suggested a relation between long term statin use and increased risk of nerve damage or polyneuropathy (Gaist et al., 2002). Other possible adverse effects include cognitive loss, neuropathy, pancreatic and hepatic dysfunction, and sexual dysfunction (Golomb & Evans, 2008).

All commonly used statins show somewhat similar results, however the newer statins, characterized by longer pharmacological half-lives and more cellular specificity, have had a better ratio of efficacy to lower adverse effect rates. Some researchers have suggested that hydrophilic statins such as fluvastatin, rosuvastatin, and pravastatin are less toxic than lipophilic statins such as atorvastatin, lovastatin, and simvastatin, but other studies have not found a connection; it is suggested that the risk of myopathy is lowest with pravastatin and fluvastatin probably because they are more hydrophillic and as a result have less muscle penetration. Lovastatin induces the expression of gene atrogin-1, which is believed to be responsible in promoting muscle fiber damage (Hanai et al., 2007).

Although there have been concerns that statins might increase cancer, several meta-analyses have found no relationship to cancer, the largest of which as of 2006 included nearly 87,000 participants (Dale et al., 2006). However, in 2007 a meta-analysis of 23 statin treatment arms with 309,506 person-years of follow-up found that there was an inverse relationship between achieved LDL-cholesterol levels and rates of newly diagnosed cancer that the authors claim requires further investigation (Alsheikh-Ali et al., 2007).

**TERMINALIA ARJUNA**

**Habitat**

*Terminalia arjuna* is a deciduous and evergreen tree, standing 20–30m above ground level (Figure 3.10). It belongs to Combretaceae family (Chopra and Ghosh, 1929; Caius et al., 1930; Nadkarni and Nadkarni, 1954). It is found in abundance throughout Indo-sub-Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi and Deccan region near ponds and rivers. It is also found in forests of Sri Lanka, Burma and Mauritius (Chopra et al., 1958).
Ethnomedical considerations

The bark, leaves and fruits of *Terminalia arjuna* have been used in indigenous system of medicine for different ailments (Warrier et al., 1996). The bark is said to be sweet, acrid, cooling and heating, aphrodisiac, expectorant, tonic, styptic, antidysenteric, purgative and laxative. Its use has been advocated in urinary discharge, strangury, leucoderma, anaemia, hyperhidrosis, asthma and tumours. The use of bark powder as an astringent and diuretic finds mention in the literature (Dwivedi, 2007). The bark powder has been attributed to possess cardioprotective properties.

Phytochemistry

As the bark was considered to be the most important constituent from medicinal point of view, most of the early studies were limited to bark stem of the plant. Chemical analysis of the bark showed evidence of sugar, tannins (12%), colouring matter, a glycoside, and carbonates of calcium, sodium and traces of chloride of alkali metals (Ghoshal, 1909). Subsequently presence of an alkaloid as well as a glycoside was confirmed. The major chemical constituents of various parts of *T. arjuna* are shown in Table 3.2). The glycoside was capable of increasing the force of contraction of the frog heart (Ghosh, 1926). Attempt to isolate the glycoside resulted into finding of an organic acid with a high melting point, a phytosterol, an organic ester easily hydrolysed by mineral acids, 12% tannins consisting largely of pyrocatechol tannins, large quantities of calcium and smaller amounts of aluminium and magnesium salts, colouring matter and sugar (Chopra and Ghosh, 1929).
Table 3.2: Major chemical constituents of various parts of *Terminalia arjuna*.

(A) Stem bark

1. **Triterpenoids**: arjunin, arjunic acid, arjunolic acid, arjungenin, terminic acid (Honda et al., 1976a; Anjaneyulu and Prasad, 1983)

2. **Glycosides**: arjunctin, arjunoside I, arjunoside II, arjunaphthanoloside, terminoside A (Ghoshal, 1909; Ghosh, 1926)

3. **Sitosterol** (Ghosh, 1926; Anjaneyulu and Prasad, 1983)

4. **Flavonoids**: arjunolone, arjunone, bicalein, luteolin, gallic acid, ethyl gallate, quercetin, kempferol, pelargonidin, oligomeric proanthocyanidins (Sharma et al., 1982; Pettit et al., 1996)

5. **Tanins**: pyrocatechols, punicallin, punicalagin, terchebulan, terflavin C, castalagin, casuariin, casuarinin (Ghoshal, 1909; Chopra and Ghosh, 1929; Lin et al., 2001)

6. **Minerals/trace elements**: calcium, aluminium, magnesium, silica, zinc, copper (Dwivedi and Udupa, 1989)

(B) Roots

1. **Sitosterol** (Anjaneyulu and Prasad, 1983)

2. **Triterpenoids**: arjunic acid, arjunolic acid, oleanolic acid, terminic acid (Anjaneyulu and Prasad, 1983)

3. **Glycosides**: arjunoside I, arjunoside II, arjunoside III, arjunoside IV, 2,19-dihydroxy-3-oxo-olean-12-en28-oic acid28-O-d-glucopyranoside (Anjaneyulu and Prasad, 1982a,b; Choubey and Srivastava, 2001)

(C) Leaves and fruits

1. **Glycosides**

2. **Flavonoids**: luteolin (Pettit et al., 1996)
**Actions of *Terminalia arjuna***

*Terminalia arjuna* (Arjuna) is an important medicinal plant widely used in medicinal formulations for several ailments. The use of *Terminalia arjuna* bark in the management of hypercholesterolaemia has been widely reported (Chander et al., 2004). Various pharmacological studies have shown its antiviral, anti-mutagenic, antiplaque formation, anticancer and hypotensive properties (Diwedi et al., 1997). Arjunolic acid, a new triterpene and a potent extract from the bark of *Terminalia arjuna*, has been shown to provide significant cardiac protection in myocardial necrosis in rats. Arjunolic acid treatment prevents the decrease in the levels of powerful antioxidants such as superoxide dismutase, catalase, glutathione, alpha-tocopherol, and ascorbic acid. It is useful as an anti-ischemic and cardioprotective agent in hypertension and in ischemic heart disease especially in disturbed cardiac rhythm, angina or myocardial infarction (Vaidya, 1994; Gupta et al., 2001). The bark powder possesses diuretic and a general tonic effect in cases of cirrhosis of the liver, in addition to prostaglandin enhancing and coronary risk factor modulating properties. It induces a drug-dependent decrease in blood pressure and heart rate. Additional actions of Arjuna include protection against DNA damage from toxins. *Terminalia Arjuna*, compared to placebo, was associated with improvement in symptoms and signs of heart failure, improvement in NYHA Class, decrease in echo-left ventricular enddiastolic and endsystolic volume indices, increase in left ventricular stroke volume index and increase in left ventricular ejection fractions (Bharani et al 2002).

Experimental studies have revealed its bark exerting significant inotropic and hypotensive effect, increasing coronary artery flow and protecting myocardium against ischemic damage. It has also been detected to have mild diuretic, antithrombotic, prostaglandin E2 enhancing and hypolipidaemic activity. However, toxicological studies in experimental animals are lacking. Considering its anti-ischemic activity and its potential to correct dyslipidemia, reduce left ventricular mass and increase left ventricular ejection fraction, it is essential to examine the molecular mechanism of its action and its core constituents. Table 3.3 compiles the various pharmacological studies on *Terminalia arjuna* related to cardiovascular system.
### Table 3.3: Pharmacological studies on *Terminalia arjuna* related to cardiovascular system.

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<th>Animal model</th>
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<td></td>
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<td></td>
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<td>Aqueous as well as alcoholic bark extract</td>
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<tr>
<td></td>
<td>Aqueous extract of bark powder in doses of 30 mg/kg</td>
<td>Isolated rat atria</td>
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<td></td>
<td>Aqueous extract of bark powder in doses of 30 mg/kg</td>
<td>Isolated rat atria</td>
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<td></td>
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<tr>
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<td><strong>Hypotensive actions</strong></td>
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<td></td>
<td>Aqueous extract of the bark, intravenously</td>
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<td></td>
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<tr>
<td></td>
<td>Aqueous extract of bark, 40 mg/kg, i.v.</td>
<td>Dog, in vivo study</td>
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</tr>
<tr>
<td>Study pertaining</td>
<td>Plant preparation dosage and route</td>
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<tr>
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<td>Bark powder 500 mg twice daily, orally in suspension form for 90 days</td>
<td>Rabbit, in vivo study</td>
<td>Aortic ring PGE2 levels increased in rabbits receiving <em>Terminalia arjuna</em> (Dwivedi et al., 1987)</td>
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<tr>
<td><strong>Cardioprotective and antioxidant activities</strong></td>
<td>Capsules containing powder of <em>Terminalia arjuna</em> bark, root of <em>Inula racemosa</em>, root of <em>Saussurea lappa</em> Clarke 500 mg twice daily administered separately orally as suspension through gavage to rabbits for 90 days</td>
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<tr>
<td><em>Terminalia arjuna</em> in the doses of 30 mg per tablet in a multimineral herbal formulation, abana, administered orally as a suspension</td>
<td>Rats subjected to myocardial ischemia induced by isoproterenol and treated with abana</td>
<td>The reversal of cardiac injury enzyme and improved heart mitochondrial uptake (Tandon et al., 1995)</td>
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<tr>
<td>Arjunolic acid derived from <em>Terminalia arjuna</em> bark extract 15 mg/kg, given intraperitoneally</td>
<td>Rats subjected to isoproterenol-induced myocardial ischemia and administered arjunolic acid both pre and post isoproterenol administration</td>
<td>Arjunolic acid treated rats had significant diminished levels of cardiac injury enzymes and raised SOD, CAT, GPx and myeloperoxidase (Sumitra et al., 2001)</td>
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<tr>
<td><em>Terminalia arjuna</em> extract in doses of 5 mg/kg</td>
<td>In vitro study</td>
<td>Ameliorate glycation of Hb and exerts antioxidant effects (Kedlaya and Udupa, 1997)</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract of <em>Terminalia arjuna</em> bark 50 mg/kg orally for 1 week</td>
<td>Mice challenged with carbon tetrachloride, 1 ml/kg body weight liver and renal enzyme markers assessed</td>
<td><em>Terminalia arjuna</em> prevented the rise in liver injury enzymes, i.e., SGPT, ALP and TBARS and increased the levels of SOD, CAT, and GSH. Results were comparable to vitamin C group mice (Manna et al., 2006)</td>
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<tr>
<td>Ethanolic extract of <em>Terminalia arjuna</em> bark in 400 mg/kg, post orally for 28 days</td>
<td>Single injection of N-nitrosodimethylamine-induced liver cancer in male rats treated with <em>Terminalia arjuna</em></td>
<td><em>Terminalia arjuna</em> treated rats demonstrated decreased levels of lipid peroxidase and near normal levels of antioxidant enzymes—SOD, CAT and glutathione peroxidase (Sivalokanathan et al., 2006)</td>
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</tbody>
</table>
### Study pertaining

<table>
<thead>
<tr>
<th>Study design</th>
<th>Observations</th>
</tr>
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<tbody>
<tr>
<td><strong>Effects on lipids</strong></td>
<td></td>
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<tr>
<td><em>Terminalia arjuna</em> bark powder 250 mg/kg administered orally twice daily</td>
<td>(a) Reduction in total cholesterol and triglycerides; (b) increase in HDL-cholesterol (Tiwari et al., 1990)</td>
</tr>
<tr>
<td>Ethanolic extract of bark in 100–500 mg/kg dose orally</td>
<td>(a) Reduces hyperlipidemia; (b) no change in HDL-chol. (Ram et al., 1997)</td>
</tr>
<tr>
<td>Ethanolic extract of the <em>Terminalia arjuna</em> Wight &amp; Arn., <em>Terminalia bellerica</em> Roxb. and <em>Terminalia chebula</em> Willd. administered orally</td>
<td><em>Terminalia arjuna</em> proved to be most potent hypolipidaemic agent, raised HDL-chol. and inhibited aortic atherosclerosis (Shaila et al., 1998)</td>
</tr>
</tbody>
</table>

**Note:** All experiments were carried out with bark constituents of *Terminalia arjuna*. Abbreviations: ALP, alkaline phosphatase; CAT, catalase; chol, cholesterol; GPx, glutathione peroxidase; Hb, haemoglobin; MPO, myeloperoxidase; SOD, superoxide dismutase; TBA RS, thiobarbituric acid reactive substances.

### Future strategies

The efficacy of *Terminalia arjuna* as a cardioprotective agent, a potent antioxidant preventing LDL cholesterol oxidation, and its potential to reduce atherogenic lipid levels have been amply demonstrated in various experimental and clinical studies. Its ability to improve autonomic control and myocardial functions in diabetes induced cardiomyopathy needs to be determined. Its impact on inflammatory and immunological markers, lipid biosynthesis, platelet aggregation, vascular reactivity and molecular actions in different cells of the cardiovascular system are few of the points which need to be addressed. This herbal drug with multiple beneficial effects in combating diabetes and the related complications without causing side effects can modulate the existing treatment strategies.