CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of mortality in many parts of the world. Ischemic heart disease and stroke are the two most common causes of death worldwide (Murray et al., 1996). Over 80 per cent of disability from cardiovascular disease occurs in low and middle income countries such as China, Russia, Poland, Mauritius, Argentina and India (Yusuf et al., 2000; Leeder et al., 2004). According to the American Heart Association, 37 per cent of Americans suffered from some form of cardiovascular disease, which accounted for 36 per cent of all deaths, with a staggering 2400 people dying daily. CVD is the leading cause of death in the United States. CVD is one of the leading causes of death globally. According to the World Health Organization (WHO) estimates 17.5 million people around the world died due to cardiovascular disease in 2005, which corresponds to 30 percent of the total number of deaths (Reddy, 2004). The Indian sub-continent (including India, Pakistan, Bangladesh, Sri Lanka and Nepal) is home to 20 per cent of the world’s population and may be one of the regions with the highest burden of CVD in the world (Anand et al., 2000; Yusuf et al., 2000). Although studies have documented that immigrants from the sub-continent living in western countries have a higher burden of CVD than other
ethnicities, less attention has been paid to the enormous burden of CVD in the Indian subcontinent itself (McKeigue et al., 1989; Enas et al., 1992).

The absence of reliable mortality data in the Indian subcontinent has necessitated estimates of the CVD burden based on cross-sectional studies that have been well described previously (Reddy, et al., 2005; Gupta, 2005). The prevalence of Coronary Heart Disease (CHD) in India was estimated to be 3 to 4 per cent in rural areas, and 8 to 10 per cent in urban areas with a total of 29.8 million affected (14.1 million in urban areas, and 15.7 million in rural areas) according to population-based cross-sectional surveys in 2003 (Gupta, 2004). By 2020, heart disease and stroke will become the leading cause of death and disability worldwide, with the number of fatalities projected to exceed 20 million a year and to more than 24 million a year by 2030 (Atlas of heart disease and stroke, 2004). In developing countries, half of all deaths of women over 50 are due to heart disease and stroke. By 2040, women in the study countries (Russia, Brazil, India, China, and South Africa) will represent a higher proportion of CVD deaths than men (World Heart Federation Fact-Sheet, 2002). Despite more understanding about the etiology and pathophysiology of CVD, the burden of CVD is likely to worsen rather than improve over the next 20 years. In terms of global burden of disease in 1999, the WHO placed myocardial infarction in sixth place and stroke in seventh place, but by 2020 they will have moved to first and fourth respectively (WHO, 2004). It is often assumed that myocardial infarction is a disease of affluent and industrialized countries. However, 80 percent of these deaths occur in low-to-middle income countries of varying size such as China, Russia, Poland,
Mauritius, Argentina and India (Bonow, 2002). The incidence of myocardial infarction is also high among people with Indian origins who are living abroad (Gupta and Gupta, 1996).

The huge burden of CVD in the Indian sub-continent is the consequence of the large population and the high prevalence of population the CVD risk factors (Reddy et al., 2005). Urbanization is characterized by marked increase in the level of psychosocial stress, all of which promote the development of dyslipidemia, hypertension and dysglycaemia (Yusuf et al., 2001).

**Physiology of CVD**

CVD is a common term for disease that affects the heart or other parts of the vascular system in the body. The cardiovascular system consists of three anatomical components the autonomous nervous, the heart and the vasculature. The three components interact in a complex manner to control blood flow to organs throughout the body. The autonomic nervous system controls a variety of body functions including blood pressure and heart rate. The heart is responsible for pumping blood through the circulating system where as the vasculature consists of the blood vessels responsible for distributing blood to various tissues of the body (Lilly et al., 2003).

**Autonomous Nervous System**

The autonomous nervous system is widely distributed throughout the body and controls a variety of body functions including blood pressure and heart rate. The
efferent peripheral autonomic nervous system is composed of two opposing subsystems, the sympathetic nervous system and the parasympathetic nervous system (Hopkins et al., 1981).

**Sympathetic Nervous system**

The sympathetic nervous system diffuses and innervates many components of the CVD. The primary neurotransmitter of post ganglionic systemic nerve fibers is nor-epinephrine also referred as nor adrenaline and these fibers are known as adrenergic fibers. The adrenal medulla is also a component of the sympathetic nervous system that is analogous to postganglionic sympathetic nerve fibers. Instead of nor-epinephrine, the adrenal medulla releases epinephrine (Adrenaline). The target organs of sympathetic nerves contain receptors for nor-epinephrine. These receptors are known as adrenergic receptors. A list of important target organs of sympathetic nervous system, the response of the organs to sympathetic stimulation, and the specific adrenergic receptor subtypes found on these target organs are:

- Vascular smooth muscle (increased contraction of skin, renal splanchnic, skeletal muscle, blood vessels, α-adrenergic receptor; increased relaxation of skeletal muscle, blood vessels via β-adrenergic receptors).
- Heart (increased contractility via β₁-adrenergic and β₂-adrenergic; increased heart rate via β₁-adrenergic receptors).
- Kidney (increased renin release via β₁ adrenergic).
- Bronchiolar smooth muscle (increased relaxation via β₂ adrenergic)
**Parasympathetic Nervous System**

The sympathetic branch sends the vagus nerve to the heart, where it releases acetylcholine causing the heart to beat more slowly and with less force. Parasympathetic nervous system can bind to receptors in the endothelium (the cells that line blood vessels) causing the release of acetylcholine, which binds to muscarine cholinergic receptors on target tissues. The effects of parasympathetic stimulation on these targets, and the major cholinergic muscarine receptor subtypes on each target tissue are:

- Heart-sinus (SA) node and AV junction (decreased heart rate-cholinergic M$_2$).
- Endothelium-(releases EDRF in response to acetylcholine-cholinergic M$_3$ which relaxes vascular smooth muscle (Hopkins, 1981).

**Anatomy of heart**

The heart is enclosed in a sac called pericardium. The cardiac wall is composed of three layers - the outer epicardium myocardium and the inner endocardium. The heart has four internal chambers termed as the right and left atria and the two lower chambers known as the right and left ventricles as shown in Fig 1. Each atria is connected to the ventricle through a muscular atrioventricular valve (AV) termed as mitral valve on the left side and the tricuspid valve on the right side. The right ventricle pumps blood into the pulmonary artery through a passive three-section pulmonary valve. The left ventricle pumps blood into the aorta through passive three-section aortic valve. The right and left coronary arteries begin in two cusps of aortic
valves and smaller arteries branch and enter the myocardium, thereby supplying oxygenated blood to its muscle fiber, (Teitz, 1976).

The myocardium contains bundle of striated muscle fibers. The alternating contraction and relaxation of these fibers generate the pumping action of heart (Cummins et al., 1974). The fibers are composed of the cardiac-specific contractile proteins actin and myosin, regulatory proteins—troponin and tropomyosin (Davidson, 2006).

**Vasculature**

The vasculature consists of the blood vessels responsible for distributing blood to various tissues of the body. The circulatory system consists of two separate circuits. In pulmonary circulation the right heart pumps blood into the pulmonary circulation and in systemic circulation the left heart pumps blood into the systemic circulation. Blood vessel controls the blood pressure. The major blood vessels controlling blood pressure are referred to as the resistance vessels and the capacitance vessels (Davidson, 2006).
Anatomy of heart

Fig. 1. Anatomy of heart
Pathophysiology of CVD:

The following is a list of some of the cardiovascular diseases that contribute to the most of the deaths:

- Angina pectoris
- Atherosclerosis
- Myocardial infarction
- Coronary artery disease
- Heart failure
- Hypertension
- Peripheral vascular disease.

- **Angina pectoris**

  Angina pectoris is often accompanied by symptoms of crushing, diffuse pain in the chest (directly over the heart), a shortness of breath that lead to gasping, weakness, anxiety and nausea and sweating. The coronary vessels narrow temporarily causing the heart muscle to suffer ischemia.

- **Atherosclerosis**

  Atherosclerosis may manifest as coronary heart disease (e.g., angina pectoris, Myocardial infarction, and sudden death), cerebrovascular disease (e.g., stroke and transient ischemic attack) or Peripheral vascular disease (e.g., circulation and critical limb ischemia). The entities often co-exist and the pathogenesis of the diseases is similar. Atherosclerosis can occur in any artery. Most commonly it occurs in aorta, the
artery that receives blood directly from the heart. Since aorta is the largest artery in the body, it is critically narrowed by atheromas.

**Atheroma Formation**

Atherosclerosis refers to the formation and hardening of fatty plaques on the inner surface of the arteries. In atherosclerosis, the arteries not only harden, they narrow, and sometimes any blood can't get through and are easily blocked by constriction or objects in the blood streams.

The internal surface of an artery is covered with a single layer of endothelial cells that are pressed against each other. Atherosclerosis is initiated by damage to the vascular endothelium. Endothelium derived relaxing factor (EDRF) is produced by the vascular endothelium which is an important mediator of vasodilator responses induced by pharmacological agents. EDRF has been identified as nitric oxide. Endothelium seems to produce continuously small amounts of ROS, hydrogen peroxide and hydrolytic enzymes. Superoxide that reacts with nitric oxide is also produced by macrophages.

Fatty streaks tend to occur at sites of altered arterial shear stress, and are associated with abnormal endothelial function. They develop when inflammatory cells, predominantly monocytes, bind to the receptors expressed by endothelial cells, migrate into the intima, take up the oxidized LDL from the plasma and become lipid-laden "foam cells" or macrophages. In response to the cytokines and growth factors produced by the activated macrophages, smooth muscle cells migrate from the media of the arterial wall into the intima and change from a contractile to a repair phenotype in an
attempt to stabilize atherosclerotic lesion. Then the lipid core will be covered by smooth muscle cell and matrix, producing a stable atherosclerotic plaque.

In an established atherosclerotic plaque macrophages mediated inflammation and smooth muscle cells promote repair. If inflammation predominates, the plaque becomes active or unstable and may be complicated by ulceration and superadded thrombosis. Activated macrophages release cytokines such as interleukin-I, tumor necrosis factor-alpha, interferon-gamma, platelet-derived growth factors and matrix metalloproteins and may cause the intimal smooth cells overlying the plaques to become senescent resulting in thinning of the protective fibrous caps; they may also digest collagen cross-struts within the plaques. These changes make the lesion vulnerable to the effects of mechanical stress and may lead to erosion, fissuring or rupture of the plaque surface. Any breach in the integrity of the plaque will expose its contents to circulating blood and may trigger platelet aggregation and thrombosis that extends into the atheromatous plaques and the arterial lumen. This type of plaques event may cause partial or complete obstruction at the site of the lesion and/or distal embolisation resulting in infarction or ischemia of the many of the acute manifestation of atherosclerotic vascular disease, (e.g., Myocardial infarction, acute lower limb and stroke) (Davidson, 2006).

- **Myocardial Infarction**

  Acute myocardial infarction (AMI) occurs during the period when circulation to a region of heart is obstructed and necrosis ensues. AMI is characterized by severe pain (angina pectoris), frequently associated with nausea, shortness of breath and
dizziness. A precursor state of AMI is myocardial ischemia, in which obstruction of a coronary artery leads to severe oxygen deprivation of the myocardium before necrosis.

The major causes of AMI are atherosclerotic plaque rupture and thrombus formation. Myocardial ischemia and subsequent infarction usually begin in the endocardium and spread towards the epicardium. When the necrosis occurs through the full thickness of myocardium, the infarct is termed transmural.

- **Heart failure**

  Heart failure, often called Congestive Heart failure, is a condition in which the heart can’t pump enough blood to body’s organs and tissues. It doesn’t mean heart has failed and can’t pump at all.

- **Coronary Heart Disease (CHD)**

  It refers to atherosclerosis of the arteries that nourish the heart muscle. CHD is a major risk factor for heart attack since narrowed coronary artery can’t provide adequate oxygen to the heart.

- **Hypertension:**

  Hypertension means that the pressure of blood exerts against the walls of the blood vessels which is dangerously high. This excess pressure weakens arterial walls
and decreases their elasticity and forcing the heart to pump harder. Arteries can also break due to this excess force and can cause hemorrhage (excessive bleeding).

- **Peripheral Vascular Disease (PVD)**
  
PVD refers to a range of disorders that affect the blood vessels in the legs, feet, arms or hands. The varieties of PVD are blood clots, atherosclerosis and varicose veins.

**Risk factors of CVD**

Most risk factors for heart disease are related to lifestyle and environmental factors (Grundy, 1998). Therefore, there are several risk factors for heart diseases: some are controllable, others are not. Controllable risk factors include gender; age; family history of heart disease; postmenopausal; race (African Americans, American Indians, and Mexican Americans are more likely to have heart disease than Caucasians). Still there are many risk factors that can be controlled. Changes in lifestyle can actually reduce risk for heart diseases. Controllable risk factors include smoking; low density lipoprotein (LDL), and high density lipoprotein (HDL), uncontrolled hypertension (high blood pressure), physical activity, obesity, uncontrolled diabetes, stress, anger and alcohol, high C-reactive protein values (it is only present during episodes of acute inflammation).

**Uncontrollable risk factors**

**Gender:** Coronary heart diseases are much more common in middle aged men. Women are likely to have angina than men. Younger women with heart disease often do not
have the same symptoms as their male counterparts and may be less likely to be diagnosed (Gupta et al., 2000).

**Age**: about 85% of people who die from heart disease are over the age of 65.

**Genetic factors**: Genetics also plays major role in increasing the likelihood of developing important risk factors (like diabetes and high blood pressure).

**Controllable Risk Factors**

**Smoking**

Smokers in their thirties and forties have a heart-attack rate that is five times higher than their non smokers. Cigarette smoking may be directly responsible for at least 20% of all deaths from heart disease (Grundy, 1998).

**Cholesterol and other lipids**

**Cholesterol**

Cholesterol is an essential nutrient necessary for a lot of functions. When cholesterol level rises in the blood, it can have dangerous consequences, depending on the type of cholesterol, particularly LDL cholesterol. Triglycerides may be the major troublemakers for the heart. Triglycerides interact with the HDL cholesterol in such a way that HDL levels fall as triglyceride level rise. Low HDL is known to be harmful to the heart (Rajmohan, 2000).

**Hypertension**

High blood pressure, or hypertension, has long been proved to cause coronary artery disease. A normal blood pressure reading is 120/80 mm of Hg. Blood pressure is regulated by sympathetic and parasympathetic branches of the autonomic nervous
system. Kidney can influence blood pressure by excreting more sodium and water and excreting the enzyme renin which produces angiotensin I in the blood stream.

**Role of Angiotensin converting enzyme in CVD:**

Angiotensin converting enzyme (EC 3.4.15.1, ACE) catalyses the hydrolysis of angiotensin-I to angiotensin-II. ACE and angiotensin-II are biologically active components of renin angiotensin-aldosterone system (RAS) which plays a central role in the maintenance of blood pressure and electrolyte and water homeostasis (Natesh, 2003). Distribution of ACE varies among tissues in any given mammalian species for unknown reasons. For example kidney has almost five folds more ACE activity than lungs in man (Udupa, 1993; Corvol, 1995). One of the characteristic properties of ACE is its requirement of monovalent ions particularly chloride for catalytic activity (Bunning et al., 1983). The mechanism by which chloride activates ACE has not been established clearly (Ballerman et al., 1991).

Salt restriction plays critical role in controlling hypertension and improves effectiveness of antihypertensive drugs in some cases (Weinberger, 1986). Since, the chloride is an integral part of salt, the effect of salt on blood pressure may be due to the chloride dependence of ACE. Increase in chloride ion concentration causes increase in the activity of serum ACE. Hence high salt intake may raise extracellular chloride level which causes activation of ACE resulting in excess angiotensin II formation and high blood pressure. In contrast, salt restriction may lower extracellular chloride level thus
leading to less formation of angiotensin II and hypotension. Therefore, the effect of salt on blood pressure could be due to chloride sensitivity of ACE (Roharbach, 1981).

**Sedentary life style and exercise**

Exercise has a number of effects that benefit the heart and circulation, including improving HDL cholesterol, decreasing lipid levels and reducing inflammation in the arteries (Ohlsson, 2004).

**Oxidative stress and Antioxidants in CVD**

There is evidence from recent epidemiological studies that antioxidants may reduce the risk of CVD. They are thought to offer protection against coronary heart disease, hyper tension and some forms of cancer. Low concentrations of antioxidant enzymes and high concentrations of lipid peroxides in plasma will increase the risk of coronary heart disease (Akila, D’Souza et al., 2007).

A free radical is defined as the molecular species capable of independent existence and with one or more unpaired electrons and is highly reactive and plays an important role in the pathogenesis of tissue damage in many different clinical disorders such as cancer, cardiovascular diseases, cataracts, immune system decline etc (Julicher, 1984). During the process of free radical generation iron is essential for the maintaining proper cell function and any iron overload may result in deleterious reaction such as degradation of proteins, nucleic acids and peroxidation of polyunsaturated fatty acids (PUFA) (Ward, 1995).
Oxidative stress is a condition in which oxidant molecules exert their toxic effect because of an increased production or an altered cellular mechanism of protection (Block et al., 2002), increased oxidative stress and the generation of oxygen free radicals can result in modification of LDL to oxidized LDL. That could lead to atherosclerotic lesion. Inflammation also occupies central position in all phases of atherosclerosis which is underlying cause of acute myocardial infarction (Libby, 2003). These oxygen free radicals are also capable of damaging compounds of biochemical classes including nucleic acids, proteins, lipids, lipoproteins, carbohydrates and connective tissue macromolecules (Carrol, 1987). Oxidative stresses also depress the sarcolemmal Ca²⁺ transport and result in the development of intracellular Ca²⁺ overload and ventricular dysfunction (Tappia et al., 2001). Oxidative stress has been implicated in the pathogenesis of myocardial ischemia. Therefore therapeutic interventions having antioxidant or free radical scavenging activity may exert beneficial effects against oxidative stress associated with various cardiovascular diseases including ischemic heart disease (Bandyopadhyay, 2004).

Antioxidant vitamins such as vitamin-E and powerful antioxidants protect PUFA and other components of the cell and organelle membranes from oxidation by free radicals. Vitamin-C, present in the cytosol, where it reacts with the alphatocopheroxy radical, and in the process gets oxidized to dehydroascorbic acid (Sun, 1990). Taurine is an antioxidant amino acid, found in appreciable quantities in plasma
and various tissues have antihypertensive, antiatherogenic and antioxidative effects in experimental animals (Dawson et al., 2000).

**Role of nitric oxide (NO) in CVD:**

Nitric oxide is a unique signaling molecule which was labeled as the “Molecule of the year” in 1992 (Stamler et al., 1992). NO has been found to exert negative inotropic and negative chronotropic effects on cardiac muscle cells. There is also evidence that in cardiac muscle NO release and possibly other factors from the endocardium may be involved in the beat to beat regulation of cardiac function (Shah, 1993).

The vascular endothelium has a primary regulatory role to inhibit adhesion and aggregation of platelets and other blood cells and to keep blood vessels dilated to maintain healthy blood flow (Moncoda et al., 1995). It is believed that NO is continuously produced by vascular endothelial cells which regulates blood flow and pressure. NO is also a potent inhibitor of platelet aggregation and constant release of NO from endothelial cells and is thus an important in maintaining appropriate levels of platelet adhesiveness. NO has also been found inside the platelets aggregation and adhesion (Wang et al, 1995). The endothelial generation of NO not only regulates blood pressure but also clotting. Endothelial dysfunction may also cause local deficiency of NO, which leads to platelet aggregation and subsequent development of atherosclerosis (Marsden, *et al*, 1993; Nadaud, 1994). Ischemia and reperfusion cause vasodilation only in affected tissue and this response is probably mediated by NO as shear stress and
increase in blood flow through a vessel are the physical stimuli to which the endothelial cells respond by increasing the NO production (Yashimura et al., Yashimura et al., 1998, 2000). Base level of NO regulates blood flow in the brain, heart, lungs, gastrointestinal tract and kidneys. Thus, NO is an endogenous autoregulator of blood flow (Stangl et al., 2000).

**Role of carnitine in CVD:**

Carnitine (CA, β-hydroxy-γ-trimethylammonium butyric acid) is synthesized in tissues such as liver, kidney and brain. Essential amino acids e.g., lysine and methionine and vitamins such as niacin, pyridoxine and ascorbic acid are required for its biosynthesis. Physiologically CA plays an important role in the transformation of long chain free fatty acids into acyl carnitine in their transport across the inner mitochondrial membrane into the mitochondrial matrix for β-oxidation which offers protection against myocardial infarction induced by ISO. Myocardial ischemia has been found to be associated with massive loading of mitochondria with calcium, which interferes with mitochondrial function and produces cellular injury (Reznick et al., 1992).

Carnitine also plays a role in chelating free Fe\(^{2+}\) ions and hence could reduce free radical generation which requires the presence of Fe\(^{2+}\). The protective effect of carnitine is probably achieved by decreasing the levels of fatty acids and peroxides and by activating the enzyme glutathione peroxidase. In addition, carnitine also decreases the level of taurine which results in decreased uptake of calcium by the myocardium.
and thereby preventing the myocardial cells to be overloaded by calcium in the normal heart (Dolara et al., 1978).

**Role of calcium (Ca\(^{2+}\)) in CVD:**

Calcium is critical in the contractile process. Extracellular Ca\(^{2+}\) plays an important role in the contraction of cardiac muscle which is rich in ion channels. The level of free intracellular calcium is restricted by the ion channels. Changes in calcium concentrations have a great effect on normal cardiac function. Calcium is thought to inhibit intracellular energy flow. When free calcium is present in high concentrations in the heart cells, it accumulates in the mitochondria, and causes uncoupling of oxidative phosphorylation and leads to decreased ATP production. In resting myocytes, Ca\(^{2+}\)-Na\(^{+}\) exchanger maintains a low levels of free intracellular calcium which contributes to relaxation but run in the reverse direction during excitation (Murray, 2000).

Tropomyosin and troponin complex located in the T-filament of cardiac muscle regulate the contraction according to the intracellular calcium. Calcium antagonists are reported to selectively block the slow calcium channels thereby exerting negative cardio protective effect on heart (Roy, 2002). Calcium movements into the cardiac cell occur via the sarcolemmal and largely are regulated by uptake and release of calcium through the sarcoplasmic reticulum. These subcellular structures regulate muscle contraction and relaxation (Buja et al., 1994).
Ca\textsuperscript{2+} ions are essential for cardiomyocytes contraction by binding to the sarcomeric protein troponin-C which alleviates its inhibition of actin-myosin interactions and allows movement of the myosin-containing thick filaments along action-containing thin filaments (Cheen, 2000; Kiriazis, 2000; Seidman, 2001). Cardiac contraction-relaxation cycles are initiated by activation of plasma membrane voltage-dependent L-type Ca\textsuperscript{2+} channels, which in turn stimulates the massive release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum (SR) into the cytoplasm by the ryanodine receptors (sarcoplasmic reticulum release channels). Ca\textsuperscript{2+} also plays a crucial role in regulating cardiomyocytes growth (Kane, 1981).

**Role of lipoproteins and apolipoproteins CVD**

Lipoproteins are complex aggregates of lipids and proteins that render the lipids compatible with the aqueous environment of body fluids and enable their transport throughout the body. The most abundant lipid constituents are triacylglycerols, free cholesterol, cholesterol esters and phospholipids (phosphatidylcholine and sphingomyelin especially). Apo proteins are required to solubilize the non-polar lipids in the circulation. The physical properties of lipoproteins contain a number of important enzymes, including lipases, acryl transferees and transport proteins.

Lipoproteins are the macromolecular complexes containing lipids and their inner core surrounded by proteins, act as carrier molecule for the insoluble lipids in plasma. Physical properties and lipid compositions of various lipoprotein classes have different classes. Apolipoproteins are the protein components of lipoproteins. Apolipoprotein
isolated from animal species are generally similar to those of the humans in their physical and biological significance and in their distribution among lipoproteins.

Lipoprotein-a (Lp-a) is a modified form of LDL that contains apoB-100 linked by a disulphide bridge to a highly polymorphic glycoprotein, apolipoprotein-B. Elevated levels of Lp-a have been linked to an increased risk of CAD and other forms of vascular disease especially when associated with the elevated LDL or reduced HDL cholesterol level. Increased concentration of Lp-a in plasma have been associated with an increased risk of the development of premature cardiovascular disease. Lp-a contributes to atherosclerosis. Pathogenecity of Lp-a also includes inhibition of transforming growth factor-β, destabilization of plaque, increased smooth muscle cell proliferation and migration, formation of occlusive thrombus, impaired formation of collateral vessels, enhanced oxidation uptake and retention of LDL-C. The two major apolipoproteins of HDL are Apo-A₁ and A₁₁ which have cardio protective role, whereas Apo-A₁₁ is associated with increased risk of atherosclerosis in animal models. Apo-A₁ activates the lecithin cholesterol of HDL perhaps by stimulating hepatic lipase (HL). Defects in the structure of Apo-B or in the assembly of Apo-B containing lipoproteins result in the failure of intestinal and hepatogeneous triglyceride rich lipoproteins to be secreted. Apo B-48 is virtually the only apolipoprotein on LDL. Both the lipoproteins are required as structural lipoprotein particles for the secretion of arginine rich lipoproteins from the intestine and liver. Abnormalities of apolipoprotein synthesis including high apo-B100, high Lp-a and low apo-A₁ levels have been reported in CHD cases (Gupta et al., 2008).
**Antihyperlipoproteinemiac Drugs**

The hyperlipoproteinemias is a condition in which the concentration of cholesterol or triglyceride – carrying in plasma exceeded in arbitrary normal units. Administrations of drugs lower plasma concentrations of lipoproteins, either by diminishing the production of lipoproteins or by enhancing the efficiency of their removal from plasma. The combination of nicotinic acid and a bile acid binding resin lowers LDL concentrations (Kane et al., 1981). Clofibrate, Gemfibrozil, Probucol, Cholestyramine and Colestipol Compactin and Mevinolin, are the drugs extremely effective in lowering plasma concentrations of LDL cholesterol with minimal toxicity.

**Plant drugs as cardiotonic compounds**

Malhotra et al. (1970) reported the importance of guggulipid as antihyperlipidaemic drug. Solar guggal, the oleoresin of Boswellia serata has been reported as an antiarthritic and antihyperlipidaemic agent. Their mechanism of action is attributed to the presence of disulphides and sulphone substance.

**Role of troponin in CVD:**

Troponin is a protein complex located on the thin filament of striated muscles consisting of the three subunits namely Troponin T (TnT), Troponin I (TnI) and Troponin C (TnC) each having different structure and function. Of the three troponins, TnT and TnI are being used as the biochemical markers for the diagnosis of myocardial injury. The troponins found in cardiac tissue (cTn) have a different amino acid sequence than that present in troponin of skeletal muscles. This makes cTnT and cTnI
more specific for the diagnosis of myocardial injury. These cardiac troponins (cTns) appear in the blood as early as 3-4 hours of the acute episode and remain elevated for 4-14 days. The pattern of release of troponin may be monophasic or biphasic. This release kinetics are related to the distribution of these proteins within the myocardial cells. About 94-97% of these troponins are bound to myofibril and only 3% of cTnI and 6% of cTnT is free in the cytoplasm (Adams, 1994).

When the myocardial damage occurs the cytosolic troponins reach the bloodstream quickly resulting in a rapid peak of serum troponin observed during the first few hours. cTnT and cTnI are now regarded as the most specific biochemical markers of myocardial injury. Studies have shown that cardiac troponins should replace CK-MB as the diagnostic 'gold standard' for the diagnosis of myocardial injury (Tymchak, 1997) the reasons being:

1. Troponins are highly cardio specific especially the TnI (100%).
2. The prolonged elevation (4-14 days) makes it a good marker for the patients admitted to the hospital after several days of MI.
3. cTns have greater sensitivity for minor degrees of myocardial injury (Hamm, 1997; Rottbauer 2002) due to the cardio-specificity and their very low concentration in serum of normal individuals.
4. These are excellent prognostic indicator in patients with unstable angina, (Ravikilde., 1993; Wu, 1999) and is a very useful parameter for stratifying risk in acute coronary syndrome (ACS) patients (Ohman, 1996; Galvani, 1997; Olatidoye, 1998; Heidenreich, 2001) and their predictive value is superior to that of CK-MB alone (Rao, 2003).
5. A single measurement of serum cTnT at the time corresponding to the slow continuous release after AMI (~72hrs after onset) can be used as a convenient and cost effective non-invasive estimate of infarct size where as CK-MB requires repetitive sampling (Zimmerman, 1999).

6. The early serial measurement of CTnl is more accurate predictor of early coronary artery reperfusion after thrombolytic therapy as compared to CK-MB and myoglobin (Apple, 1996).

7. According to U.S. National Academy of Clinical Biochemistry (NACB) and joint European Society of Cardiology and American College of Cardiology (ESC/ACC) guidelines, cTns are the most of most specific and sensitive biochemical markers (Wu, 1999; Alpert, 2000).

**Myoglobin in CVD:**

Myoglobin, a 18 KD cytosolic protein, appears in blood earlier after myocardial injury than any other marker available so far. The detectable levels of myoglobin in the blood are found as early as 2 to 3 hours after the onset. Its peak value is obtained at 6 – 12 hours after the onset of the symptoms and then it normalizes over the next 24 hours. However, it is not cardiac specific as its release from the skeletal muscles cannot be distinguished from that released due to cardiac injury (Christenson,1998) and it is found to be elevated in severe renal insufficiency and in patients being on alcohol binges immediately prior to coming to emergency room (Gilkeson, 1978).

Carbonic anhydrase III (CA III) is present in skeletal muscles and is released into circulation following injury. But since it is not found in cardiac muscles it is used
in combination with myoglobin as a marker of myocardial damage (Vannanen, 1990). The measurement of myoglobin / carbonic anhydrase III (CA 111) ratio improves the specificity of myoglobin as an early marker of MI (Brogan, 1996). The ratio was found significantly higher in patients with MI whereas myoglobin and CA III were released in a fixed ratio following exercise and showed no significant difference in the ratio for trauma patients (Beuerle, 2000).

**Heart-Type Fatty Acid Binding Protein (H-FABP) in CVD**

It is a low molecular weight (15KD) cytoplasmic protein present in myocardium and is released into the circulation following myocardial injury. Its plasma kinetics closely resemble those of myoglobin but it is more cardio specific than Myoglobin. It is found to be elevated within 3hr after AMI and returns to normal level within 12-24hr. Hence it is considered as a sensitive and specific marker of early detection of myocardial injury as compared to CK-MB and/or α-HBDH and Myoglobin (Kleine, 1992; Ishii, 1997; Okamoto, 2000). It also has a potential to be used as a potential to be used as a prognostic indicator of myocardial damage as well as clinical outcome in pediatric cardiac surgery (Hasegawa, 2004).

**Isoproterenol**

Isoproterenol [(1,(3,4) dihydroxyphenyl- 2-isopropylamino ethanol) hydrochloride] (ISO) is a synthetic catecholamine and beta-adrenergic agonist which causes severe oxidative stress in myocardium resulting in infarct like necrosis of the myocardium (Wexler, 1978).
Induction of MI:

ISO induced myocardial infarction serves as a well standardized model for studying certain physiological and pathological events i.e., changes in lipids, enzymes and hormones during the course of acute myocardial infarction (Judd, 1974). It also alters the membrane permeability, myocardial integrity, Ca\(^{2+}\) overload and insufficient oxygen utilization (Opie, 1985). Myocardial ischemia results in alterations of cardiac structure and function. This is accompanied by disruption of the mitochondria along with the inactivation of the enzymes concerned with the energy metabolism of the myocardium (Kloner, 1980). During ischemia, cellular stores of ATP and creatine phosphates are depleted, phospholipids are degraded, membrane permeabilities increased and the cytosolic levels of Na\(^{+}\) and Ca\(^{2+}\) raised. The mitochondria exhibit an altered respiration and lower respiratory control index (Saris, 1995). It has been reported to exhibit many metabolic and morphological aberrations in the heart tissue of experimental animals similar to that of human myocardial necrosis by a multiple step mechanism (Shiny, 2005). The primary disturbance of ISO-induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting in increased cAMP formation, which inturn leads to increased lipid accumulation in the myocardium (Subash, 1978). It is also well known to generate free radicals and to
stimulate lipid peroxidation, which may be a causative factor for irreversible damage to
the myocardial membrane. More recently, the role of lipid peroxidation in cell damage
has received increasing attention especially with regard to ischemia-reperfusion injury
in vivo and its potential prevention or amelioration by antioxidants and oxygen free
radical scavengers (Ithyayarasi, 1997).

**Importance of Medicinal Plants**

Medicinal plants have been the major source of drugs in Indian system of
medicine and other ancient system in the world. Medicinal plants which constitute a
segment of the flora provide raw material for use in all the indigenous system of
medicine in India namely Ayurveda, Unani, Siddha and Tibetan medicine. According
to WHO, 80 per cent of the population in developing countries relies on traditional
medicine, mostly in the form of plant drugs for their primary health care needs.
Additionally, modern medicines contain plant derivatives to the extent of about 25 per
cent. An intact /native preparation may contain dote and antidote. Earlier descriptions
of curative properties of medicinal plants was found in Rig-Veda (2500-1800BC). Chakrasamitha and SushraSammitha give extensive description on various medicinal
herbs. Medicinal plants have the advantage of having little or no side effects. Some of
them are being used in traditional systems of medicine from hundreds of years in many
countries of the world (Xu et al., 2000).

In the practice of modern medicine, it is recognized that high blood pressure,
atherosclerosis, easy blood clotting and heart enlargement can lead to catastrophic
events such as heart attack and stroke which are the principal causes of death in persons over 40 years of age. As a result, millions of adults are taking one or more of the drugs to lower blood pressure, cholesterol, and/or to reduce platelet aggregation. Presently, the medicinal fraternity and the patients have increasingly started using plants to overcome various illness and sufferings mainly to obviate the profound side effects encountered in usage of modern drugs. Plant drugs safely interact with the free radical and terminate the chain reactions before vital molecules are damaged. The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed.

**Tribulus terrestris**

The botanical name of Gokshura is *Tribulus terrestris* and it belongs to Zygophyllaceae family. Other names for *Tribulus terrestris* in different languages are Hindi- chota- gokhru, Sanskrit - Laghu Gokshura, English - Small caltrops, Telugu – Chinnapalleru. It is a valuable herb known for its application in the folk medicine in many parts of the world (Tomova et al., 1981; Wu et al., 1999,). The Sanskrit name gokshura denotes the appearance of the fruit, which resembles the cloven hoof of a cow. It is also called as Svadukantaka, Iksugandhika (one which smells like sugarcane). Charaka has categorized it as mutra virechana (diuretic and one of the ingredients of laghu panchamula), five minor roots, namely, salaparni, prsniparni, brahati, kantakari and goksura. Susruta mentions it as balya nutritive tonic, sukra sudhana (sperm purifier). Later on in Ayurvedic scriptures it is cited as pramehaghna.
(antidiabetic), asmarighna (litho-tryptic), amavataghna (anti gout) and mutrakrchraghna (an alleviator of dysuria). Gokshura is recommended as a drug of choice for dysuria.

The plant occurs throughout India almost up to 3,000 meters altitude. It is a prostrate spreading herb, densely covered with minute hair. The shrub is annual or perennial and thrives in moist soils. The leaves are in opposite pairs, 5-8 cm long, compound and the leaflets 4-7 pairs are 8-12 mm long. The flowers are bright yellow, leaf-opposed, solitary, 1-15 cm in diameter. The fruits very characteristically are globose, consisting of five woodycoccii, each with two, paired sharp spines. Seeds, within each coccus are shown in Fig 2.

A large number of individual chemical constituents have been identified from the fruit and leaves, flavonoid components from the fruit and leaves, flavonoid components like kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside and a new acylated kaempferol-3-glucoside and their structures are shown in Fig 3 (Kirthikar et al., 1975). Furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin, protodioscin and sarsasapogenin type are frequently found in this plant (Su et al. 2009). Extracts and steroidal saponins have been found to possess various pharmacological activities. Preparations based on the saponin fraction of Tribulus terrestris are used for treatment of infertility and libido disorders in men and women as well as for treatment of cardiac diseases. Food supplements containing Tribulus terrestris extracts are on sale in USA and Europe with claim of a general stimulating action. Gokshura is a creeper and is very effective against urinary tract disorders because it promotes the flow of
urine, cools and soothes the membranes of the urinary tract and aids in the expulsion of urinary stones (Xu et al. 2000).

*Tribulus terrestris* is a well known herb used in the folk medicine of many countries for a number of diseases. The high content of steroidal saponins is a characteristic feature of this plant. Derivatives of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, ruscogenin, chlorogenin and sarsasapogenin are found. The sugar moieties of the isolated furostanol and spirostanol saponins are oligosaccharides, which contain 2–4 different kind of sugars – glucose, rhamnose, galactose and xylose.

The plant is an industrial source for production of medicinal preparations based on its saponin fraction. *Tribulus terrestris* still remains a plant object of further studies.
Fig 2. Tribulus terrestris (Linn..)
Fig. 3. Structures of important phytochemicals.

1. Kaempferol

2. Kaempferol-3-glucoside

3. Kaempferol-3-rutinoside

4. Protodioscin
The plant grows in many tropical and moderate areas of the world. Greek, used *Tribulus* for diuretic disorder. In India it is used a diuretic, antiseptic and anti-inflammatory. The Chinese used it for a variety of liver, kidney and cardiovascular diseases. It is the best panacea for infantile or hypoplastic uterus, when combined with satavari, ashwagandha and yastimadhu. A significant benefit of *Tribulus terrestris* is stimulation of hormone production to a balanced level, without overstimulating the secretion of hormones. The liver is a major synthesizer of hormones. The hormones are synthesized from cholesterol. A herb such a *Tribulus terrestris* that has a stimulating effect on the liver will have a major influence on cholesterol and other products of the liver. No adverse effects on the central nervous system or cardiovascular system were noted in any of the clinical studies. No known negative effect presently exists when *Tribulus* is used as a dietary supplement.

*Tribulus terrestris* is a natural herb used for treating many diseases including hypertension Philip et al.(2006) carried out antihypertensive and vasodilator effects of methanolic and aqueous extracts of *Tribulus terrestris* in rats (Philip et al., 2006). The aqueous extract of *Tribulus terrestris* possesses significant antihypertensive activity when compared to methanolic extract. They reported that antihypertensive effects of *Tribulus terrestris* are due to direct arterial smooth muscle relaxation possibly involving nitric oxide release and membrane hyper polarization (Sharif et al., 2003).
Liver

The liver, the largest organ in the body, weighs 1200–1500g and comprises one-fiftieth of the total adult body weight. It is relatively larger in infancy, comprising one-eighteenth of the birth weight. This is mainly due to a large left lobe.

The liver has a double blood supply. The portal vein brings venous blood from the intestines and spleen and the hepatic artery, coming from the coeliac axis, supplies the liver with arterial blood. These vessels enter the liver through a fissure, the porta hepatis, which lies far back on the inferior surface of the right lobe. Inside the porta, the portal vein and hepatic artery divide into branches to the right and left lobes, and the right and left hepatic bile ducts join to form the common hepatic duct.

The ligamentum venosum, a slender remnant of the ductus venosus of the fetus, arises from the left branch of the portal vein and fuses with the inferior vena cava at the entrance of the left hepatic vein. The ligamentum teres, a remnant of the umbilical vein of the fetus, runs in the free edge of the falciform ligament from the umbilicus to the inferior border of the liver and joins the left branch of the portal vein. Small veins accompanying it connect the portal vein with veins around the umbilicus. These become prominent when the portal venous system is obstructed inside the liver.

The venous drainage from the liver is into the right and left hepatic veins which emerge from the back of the liver and at once enter the inferior vena cava very near its point of entry into the right atrium.
The liver is completely covered with peritoneum, except in three places. It comes into direct contact with the diaphragm through the bare area which lies to the right of the fossa for the inferior vena cava. The other areas without peritoneal covering are the fossae for the inferior vena cava and gall bladder.

The liver is kept in position by peritoneal ligaments and by the intra-abdominal pressure transmitted by the tone of the muscles of the abdominal wall.

**Hepatic Necrosis in Heart Failure**

The liver is particularly sensitive to oxygen and the integrity of the liver cell depends on an adequate oxygen supply. The liver cells at the centre of the lobule receive blood at a lower oxygen tension than at the periphery (Blalock and Mason, 1936) and are therefore more susceptible to any fall in the oxygen supply to the liver. *Anoxia* is known to cause both degeneration of central liver cells (Martin, Bunting, and Loevenhart, 1916), and dilatation of sinusoids (Seneviratne, 1949).

The hepatic blood flow diminishes in proportion (Myers and Hickam, 1948). The oxygen supply to the liver decreases with diminishing cardiac output. Centrilobular hepatic necrosis in heart failure ought therefore to bear some relation to the degree of depression in cardiac output, but there is a group with high cardiac output showing conspicuous centrilobular necrosis in which other factors such as low arterial oxygen saturation and anemia may be involved.
The oxygen supply to the liver depends not only on the hepatic blood flow but also on the oxygen content of the portal venous and hepatic arterial blood. A correlation between arterial oxygen unsaturation and the extent of centrilobular necrosis might be anticipated. Very little estimation was available but a positive correlation was not established. However, six patients with pulmonary heart disease with high cardiac output did have low arterial oxygen saturations. Five of these had grade B liver cell necrosis and in these subjects the reduced arterial oxygen saturation seemed an important factor in the production of the liver lesion. Increased pressure in the hepatic veins might conduce to the centrilobular necrosis. Although patients with the maximal hepatic necrosis often have high right auricular and presumably hepatic venous pressures, the correlation was not constant. The patient with constrictive pericarditis who probably had the highest hepatic venous pressure also showed very severe centrilobular necrosis, a condition found in most patients with constrictive pericarditis. As Bolton (1914) pointed out it is doubtful whether the centrilobular changes produced by "passive "venous congestion are mechanical since the rise of pressure must extend right through the lobule to the portal veins. He believed them to be due to "a nutritional disturbance and presumably oxygen lack resulting from blood flow changes." Another factor determining the extent of the necrosis is the duration of the heart failure, as shown in the present series.

Heart failure can undoubtedly produce centrilobular cirrhosis. The relative rarity of this condition is probably related to the infrequency of prolonged heart failure. Most patients die in the stage of centrilobular reticulin condensation and never pass to the
Fig. 4. Structure of the liver.

4a. Anterior view of the liver.

4b. Posterior view of the liver.
proliferative regenerative phase. Repeated episodes of failure are necessary for the development of full cardiac cirrhosis. Patients with rheumatic mitral stenosis, responding intermittently to treatment, are therefore particularly prone to develop cardiac cirrhosis. If patients with other forms of heart failure survive for a sufficient length of time they too can develop the same lesion. Although the connective tissue in heart failure is primarily centrilobular, in the later stages the portal tracts become involved. Fibrous bands link not only central areas but also portal tracts which may account for the reported frequency of portal cirrhosis in heart failure (Katzin, Waller and Blumgart, 1939). Serial hepatic biopsies show that the essential cirrhosis of heart failure is a centrilobular one and involvement of the portal tracts is merely a later confusing feature.

In induced cardiovascular diseases, hepatic damage always occurs. Liver cells contain many enzymes that may be released into the blood in various pathological processes (Zimmerman et al., 1970). Recently, herbal drugs, which are non-toxic and naturally occurring, are gaining much significance in the treatment of many diseases.

**Aims and scope**

It is reported that TTFAEt has cardiotonic and hepato protective properties. A very little information on cardiotonic and hepato protective effect of TTFAEt is available and no systemic study on these properties is reported to the best our knowledge. So, the present was undertaken to investigate cardiotonic and hepato protective effect of TTFAEt on albino Wister rats in which oxidative stress is induced by ISO.
Understanding of CVD pathology associated with liver abnormality would require a lifetime of serial studies. The present study includes:

- Analysis of alterations in the cardiac lipid profile: total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL), Low density Very low density lipoprotein (VLDL) and Lipoprotein cholesterol (LDL).

- Estimation of the concentration of serum electrolytes and minerals.

- Evaluation of the concentration of serum albumin and globulin.

- Assessment the body weights and heart weights in control and experimental rats.

- Analysis of alterations in the activities of liver marker enzymes viz, Glutamate pyruvate transaminase (GPT), Glutamate oxaloacetate transaminase (GOT) and Lactate dehydrogenase (LDH).

- Histological studies of liver tissue.