INTRODUCTION
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 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, and the World Health Organization (WHO) estimates that 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 3-4 per cent in rural areas, and 8-10 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). In 1990, there were an estimated 1.17 million deaths from CHD in India and in 2003, and the number is expected to almost double to 2.03 million by 2010 (Ghaaffer et al., 2004).

By 2020, heart disease and stroke will become the leading cause of death and disability worldwide, with the number of fatalities projected to increase to more than 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 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 understanding more 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 occurs 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 physical activity, and a heightened level of psychosocial stress, all of which promote the development of dyslipidemia, hypertension and dysglycaemia (Yusuf et al., 2001).

1.1 Physiology of CVD:

CVD is a common term for disease that affect 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 controls blood flow to organs throughout the body. The autonomic nervous system control a variety of body functions including blood pressure and heart rate. The heart is responsible for pumping blood through 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).
1.1.1 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).

1.1.1.1 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 postganglionic sympathetic nerve fibers. Instead of nor-nephrine, 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 to β-adrenergic receptors).
- Heart (increased contractility via $\beta_1$-adrenergic and $\beta_2$-adrenergic; increased heart rate via $\beta_1$-adrenergic receptors).
- Kidney (increased renin release - $\beta_1$ adrenergic).
- Bronchiolar smooth muscle (increased relaxation $\beta_2$-adrenergic)

1.1.1.2 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 acetylcholine-cholinergic M$_3$ which relaxes vascular smooth muscle (Hopkins.,1981)}
1.1.2 Heart

1.1.2.1 Anatomy

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 average adult human heart weighs approximately 3.25g in men and 2.75g in women. 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.
Fig 1: Heart
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).

1.1.3 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).
1.2 Pathophysiology of CVD:

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

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

- **Angina pectoris:**
  This is often accompanied by symptoms of crushing, diffuse pain in the chest (directly over the heart), a shortness of breath that leading to gasping, weakness, anxiety, light-headedness, 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, 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-exists 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 factors (EDRF) is produced by the vascular endothelium and 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 Superoxide, hydrogen peroxide and hydrolytic enzymes. Superoxide 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 interleukins-1, 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:**
  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 artery walls and decreases their elasticity 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.

**1.3 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; Post-menopausal; 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: Smoking; Low density lipoprotein (LDL), and High density lipoprotein (HDL); uncontrolled hypertension (high blood pressure); physical activity; obesity; uncontrolled diabetes; stress and anger; alcohol; high C-reactive protein values (it is only present during episodes of acute inflammation).

  **1.3.1 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).

1.3.2 Controllable Risk Factors:

Smoking
Smokers in their thirties and forties have a heart-attack rate that is five times higher than their nonsmokers. 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 rise 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 (Singh, 2003).
**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).

### 1.3.3 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 Ca\textsuperscript{2+} transport and result in the development of intracellular Ca\textsuperscript{2+} 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 (Young et al., 2001; Bandyopadhyay, 2004).

Lipid peroxidation occurs mainly in membranes where the content of unsaturated fatty acids is relatively high. Greater the unsaturation, greater is the lipid peroxidation. Cumulative effects of lipid peroxidation have been implicated as underlying mechanism in various pathological conditions like atherosclerosis, hemolytic anemia and ischemia (Halliwell, 1991). Peroxidation of biological membranes increases their leakiness to ions and causes damage to transmembrane proteins such as receptors and enzymes (Bast, 1993). Malondialdehyde is the major end product of the free radical reaction on membrane fatty acids. Cellular antioxidant defense systems such as superoxide dismutase (SOD), a copper/zinc containing metalloprotein removes the superoxide radical by combining it with proton to form hydrogen
peroxide and oxygen. Catalase decomposes hydrogen peroxide to water and oxygen. Glutathione peroxidase, a selenium containing metalloenzyme can remove hydrogen peroxide by converting reduced glutathione to oxidized glutathione (D'souza et al., 2008). It can also terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides from the cell membrane. Reduced glutathione is regenerated by NADPH-dependent glutathione reductase (Halliwell, 1990; Shahidi, 1992; Shrinivas, 2000).

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 alpha-tocopheroxy 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 has antihypertensive, antiatherogenic and antioxidative effects in experimental animals (Dawson et al., 2000).

1.3.4 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 is thus of 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., 1998; Yashimura et al., 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).

1.3.5 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 acylcarnitine in their transport across the inner mitochondrial membrane into the mitochondrial matrix for β-oxidation which offers protection against myocardial infarction induced by isoproterenol. 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).

CA also plays a role in chelating free Fe²⁺ ions and hence could reduce free radical generation which requires the presence of Fe²⁺. 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).

1.3.6 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 channel. 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 Ca²⁺-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 I-filament of cardiac muscle regulate the contraction according to the intracellular calcium. Calcium antagonists are reported to selectively block the slow calcium channels thereby exerts negative cardio protective effect on heart (Roy, 2002). Calcium movements into the cardiac cell occur via the sarcolemmal and largely are regulate by uptake and release of calcium through the sarcoplasmic reticulum. These subcellular structures regulate muscle contraction and relaxation (Buja et al., 1994).

Ca$^{2+}$ ions are essential for cardiomyocytes contraction by binding to the sarcomeric protein troponin-C which alleviates its inhibition of actomyosin 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$^{2+}$ channels, which in turn stimulates the massive release of ca$^{2+}$ from the sarcoplasmic reticulum (SR) into the cytoplasm by the ryanodine receptors (sarcoplasmic reticulum release channels). Ca$^{2+}$ also plays a crucial role in regulating cardiomyocytes growth (Kane, 1981).

1.3.7 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-aldosteron 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 rise 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).

1.3.8 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 (Tnl) and Troponin C (TnC) each having different structure and function. Of the three troponins, TnT and Tnl 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 cTnl 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 is related to the distribution of these proteins within the myocardial cell. About 94-97% of these troponins is bound to myofibril and only 3% of cTnl and 6% of cTnT is free in the cytoplasm (Adams, 1994).

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

1. Troponins are highly cardio specific especially the Tnl (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 (Rottbauer, 1996; Hamm, 1997), 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 , 1996) and is a very useful parameter for stratifying risk in acute coronary syndrome (ACS) patients (Galvani,
1997; Ohman, 1996; 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 (Alpert, 2000; Wu, 1999).

1.3.9 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 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).

### 1.3.10 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 cardiосpecific than myoglobin (It is found to be elevated within 3hr after AMI and returns to normal levels 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).

### 1.4 Isoproterenol

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

\[ \text{Isoproterenol} \]

1.4.1 Induction of MI:

Isoproterenol 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 isoproterenol-induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting in
increased cAMP formation, which in turn 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).

1.5 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 description of curative properties of medicinal plants was found in Rig-Veda (2500-1800BC). Chakrasamhitha and Sushra Samhitha 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 encountered in usage of modern drugs. Plant drugs safely interact with the free radical and terminate the chain reaction before vital molecules are damaged. The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed.

1.5.1 *Tribulus terrestris*

<table>
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<tr>
<th>Botanical name</th>
<th><em>Tribulus terrestris</em></th>
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<tr>
<td>Family</td>
<td>Zygophyllaceae</td>
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<tr>
<td>Parts Used</td>
<td>Fruit, Leaves</td>
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<td>Names</td>
<td>Hindi- chota-gokhru</td>
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<td>Sanskrit - Laghu Gokshura</td>
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<td>English - Small caltrops</td>
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<td>Telugu – Chinnapalleru.</td>
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The botanical name of goksur is *Tribulus terrestris* and it belongs to Zygophyllaceae family. *Tribulus terrestris* 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 goksur denotes the
appearance of the fruit, which resembles the cloven hoof of a cow. It is also
called as Svadukantaka — of sweet spines, Iksugandhika — one which smells
like sugar cane. Caraka has categorized it as mutra virecana — diuretic and
one of the ingredients of laghu pancamula — five minor roots, namely,
salaparni, prsniparni, brhati, kantakari and gokura. Susruta mentions it as
balya nutritive tonic, sukra sodhana — sperm prifier. Later on in Ayurvedic
scriptures it is cited as pramehaghna — antidiabetic, asmarighna — litho-tryptic,
amavataghna anti gout and mutrakrcchraghna — an alleviator of dysuria.
Gokura is recommended as a drug of choice for dysuria.

The plant occurs throughout India almost upto 3,000 metres 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 woodycocci, each
with two, paired sharp spines. Seeds, numerous, 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 tracts 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 (Linn.,)

A. FRUIT

B. FLOWER
Fig 3: Structures of important phytochemicals of Tribulus terrestris

- Kaempferol
- Kaempferol-3-glucoside
- Kaempferol-3-rutinoside
- Protodioscin
The plant grows in many tropical and moderate areas of the world. Greek used *Tribulus* for diuretic disorder. Indians used it as 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 over stimulating 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 to 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* posses significant antihypertensive activity than when compared to methanolic extract. They reported that antihypertensive effects of *Tribulus terrestris* direct arterial smooth muscle relaxation possibly involving nitric oxide release and membrane hyperpolarization (Sharif et al., 2003).
1.6 Aim and Scope

Understanding of CVD pathology would require a lifetime of serial studies.

The use of animal model

The present study includes:

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

- Analysis of alterations in the activities of cardiac marker enzymes in serum viz., Creatine Kinase (CK), Lactate dehydrogenase (LDH) Glutamate pyruvate transaminase (SGPT), Glutamate oxaloacetate transaminase (SGOT) and Xanthine Oxidase (XOD) in heart tissue.

- Estimate the extent of lipid peroxidation (LPO) and the levels of GSH, Antioxidant defense enzymes like Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione-S-transferase (GST) and Glutathione reductase (GR) in heart tissue.

- Histological studies of heart tissue.
REFERENCES


