CHAPTER 2
INTRODUCTION

Cardiovascular disease (CVD), remain the principal cause of death in both developed and developing countries. It may present as a typical `heart attack', as sudden death, or it may be detected at a later date and be described as a silent infarct. CVD includes high blood pressure, coronary heart disease, congestive heart failure, stroke and accounts for 17,000,000 deaths per annum worldwide. It is predicted that CVD will be the most important cause of mortality in India by 2020 (Gilski and Borkenhagen, 2005). In India the number of patients being hospitalized for heart attack is increasing over the past 35 years and male patients have shown a more striking increase.

Myocardial infarction (MI) occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue. It means necrosis of a region of myocardium caused by an interruption in the supply of blood to the heart usually as a result of occlusion of a coronary artery also called as cardiac infarction (Bono and Boon, 1992). MI is usually characterized by varying degree of chest pain, sweating, weakness, vomiting, arrhythmia and sometimes causing loss of consciousness and even sudden death. Several factors increases the risk of developing atherosclerosis and heart attack includes elevated level of low density lipoproteins and triglycerides, accompanied by reduced high density lipoproteins levels (Smith *et al*, 2004), Increased blood cholesterol, high blood pressure, use of tobacco, diabetes mellitus, male gender, family history of coronary heart disease and change in life style.

In the new millennium, CVD is looming as the new epidemic disease, affecting Indians at the relatively younger ages with severe and diffuse form of lesions. Recently, the subject of CVD in Indians has become the challenge for many researcher centers in the worldwide. The prevalence of CVD has progressively been studied from India during later half of the century particularly among the urban population. Few other studies from India are in support to have assessed the effect of urbanization and socioeconomic factors on the components of metabolic

syndromes namely hyperlipidemia, atherosclerosis, CAD, hypertension, diabetes mellitus, upper body obesity and cancer etc in different socio economic groups. Recently used antihyperlipidemic drug or drug combination lag behind the desired properties of ideal treatment. Lack of safety, efficacy, diminution in response on long term use, no guarantee of avoiding surgery, free from disease conditions and often result in patient non-compliance even in the modern era of medicine and medical sciences and developed techniques (Wincour *et al*, 1992).

Good coronary care units, the use of thrombolytic and anti-arrhythmic drugs, and accurate methods for assessing cardiac function and coronary artery pathology have all successfully reduced in-hospital mortality. However, about 75% of those who have a myocardial infarction (MI) die outside hospital (Ruston *et al*,1998), and sudden death is the first and only manifestation in about 20% of all those who present with CAD (Kannel *et al*,1984). Therefore, treatment is not available for most patients, and the aim must be to prevent the development and progression of CAD.

2.1. Free radicals, Oxidative stress and Myocardial infarction

Oxygen is vital for aerobic life processes. However, about 5% or more of the inhaled O_2 is converted to reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) by univalent reduction of oxygen (Harman, 1993). Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. When the balance between ROS production and antioxidant defenses is lost, '*oxidative stress*' results to various pathological conditions including cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all the vital organs, cancer, and premature aging (Thomas and Kalyanaraman, 1997). The free-radical-mediated oxidative stress results in

oxidation of membrane lipoproteins, glycoxidation, and oxidation of DNA, subsequently cell death results.

Various necrotic factors, proteases, and ROS from damaged cells also attack the adjacent cells, resulting ultimately in tissue injury. Furthermore, tissue injury itself has been reported to cause severe oxidative stresses. Injury caused by ischemia reperfusion, heat, trauma, freezing, severe exercise, toxins, radiation or infection, leads to the generation of ROS, and development of various disease processes (Halliwell, 1997). A growing body of evidences suggested the involvement of oxidative stress in the genesis of CVD. Damage to the myocardium cells arises due to the generation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radical and peroxynitrie (Vaage and Valen, 1993). ROS can be formed in the heart by xanthin oxidase (XO), NAD (P) H oxidases, cytochrome P450 and by uncoupling of NO synthase (NOS) (Frank, 2005) Increased production of free radical associated with decreased level of antioxidants in the myocardium, plays a major role in CVD such as myocardial infarction, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmias (Das and Maulik, 1995).

The oxidative modification of circulating lipoproteins by free radicals, particularly low-density lipoproteins (LDL), is important for the development of atherosclerosis. Smaller, denser LDL particles, which are known to be a risk factor for cardiovascular disease, may promote atherogenesis for several reasons. These modified LDL particles do not bind readily to the endogenous LDL receptor and are therefore not cleared from the circulation by this mechanism. They penetrate the arterial intima more easily, are more readily oxidized, possibly because they contain less antioxidant protection, and are taken up by the macrophage scavenger receptors, accelerating foam-cell formation. This early histological feature leads to the development of atherosclerotic plaques (Witztum and Steinberg, 1991). ROS may contribute to the remodeling processes in a number of ways, including

activating matrix metalloproteinase (MMP) that participate in reconfiguration of the extracellular matrix, acting as a signaling molecules in the development of compensatory hypertrophy and contributing to myocyte apoptosis or other cell death mechanism (Mann and Spinale,1998).

2.2. Experimentally induced myocardial infarction

Isoproterenol (ISO), a beta adrenergic agonist and a synthetic catecholamine causes sever stress in the myocardium resulting in an infarct like necrosis of heart muscle. The rat model of isoproterenol-(ISO) induced myocardial necrosis serves as a well accepted standardized model to evaluate several cardiac dysfunctions and to study the efficacy of cardioprotective agents (Rathore *et al*, 1998). A growing body of evidence is emerging which suggests that reactive oxygen derived free radicals play a crucial role in the pathogenesis of isoproterenol induced myocardial infarction. ISO induced myocardial infarction has been shown to be accompanied by hyperglycemia, hyperlipidemia, increased in serum marker enzymes and sever haemodynamic and biochemical parameters. ISO stimulates adenylate cyclase, activation of sodium and calcium channels, exaggerated calcium inflow and energy consumption, leading to cellular death (Milei *et al*, 1978). Autooxidation of ISO generates free radicals which initiate the peroxidation of membrane bound lipid leading to both functional and structural myocardial injury (Thompson and Hess, 1986).

2.3. Natural products - a promising approach

One of the major therapeutic goals of modern cardiology is to design strategies aimed at minimizing myocardial necrosis and optimizing cardiac repair following myocardial infarction. Although spectacular progress has been made in understanding the pathophysiology of myocardial infarction, nevertheless this disease remains to be the leading cause of death and disability and looking into the next millennium, researchers expect that it will continue in this position for the foreseeable future. Hence, the best course would be the identification of a

therapeutic intervention that will foreclose the hatchway to myocardial infarction.

Good health is a phenomenon rare in today's fast moving world, where people live in a stressful environment and follow an unplanned diet and unbalanced life style. It has become the need of the hour that a new vibrant medical system evolved which is devoid of any side effects and which leads resurgence of Ayurvedic traditions. Herbal and herbal-based molecules are expected to form the basis for such a development. Even in the era of genetic engineering, plants account for forty percent (40%) of all the medicinal formulation prescribed in the United States. In China about forty percent (40%) of the total medical consumption is attributed to traditional tribal medicines. About 1400 herbal preparation are used widely, according to a recent survey in member states of European Union. World Health Organization has recognized the traditional medicines as a part of the healthcare system. WHO currently encourages, recommends and promotes traditional herbal medicines in National theatre programmes due to their case of availability, low cost, safety, and people's faith in such remedies (Diwakar, 2002).

Herbal medicines are now being developed in dosage forms using modern manufacturing and processing techniques. Modern herbal research is focused mainly on activity-guided isolation (AGI) of Phytoconstituents from the crude drug. Many of the plants used in herbal medicines contain principles whose effects can be demonstrated pharmacologically and the action of whole plant extract can usually be related to that of the isolated constituents, accurate methods of assays for herbal medicines are often lacking when the active constituents are unknown and there is no means of assessing the therapeutic potency (Bhat, 1997).

2.4. Antioxidants

Anitioxidants are substances that protect cells from damage caused by free radicals. Several mechanistic studies have investigated the potential benefits of

antioxidants and therapeutics. Many recent studies have suggested that antioxidant rich foods reduce myocardial necrosis and injury induced by oxygen free radicals, despite the previously disappointing response to antioxidants still holds therapeutic promise in both treatment and prevention.

The activity of natural antioxidants is due to the presence of substituted groups such as carboxyl group, electron withdrawing group, electron donating group etc they may be phenolic or non-phenolic. In phenolic the number and position of phenolic groups decide the antioxidants potential of a compound. Hence hydroxyl group donate hydrogen to radicals, which are converted to a stable non- radical products and the chain propagation is terminated.

The total flavonoids contents remained constant during storage in both air dried and fresh spinach stored in modified atmosphere. Polyphenols with an intermediate oxidants state can exhibit higher radical scavenging activity than monooxidised polyphenols. The higher antioxidants activity of the partially polyphenols could be attributed to their increased ability to donate a hydrogen atom (Ying, 1997).

2.5 Vitamin E

Vitamin E is a fat-soluble non-enzymatic antioxidant that stops the production of ROS formed when fat undergoes oxidation. During myocardial injury caused by oxidative stress the plasma Vitamin E concentration significantly reduces and there is a deficiency of endogenous vitamin E. So, supplementation of vitamin E or its regeneration by administration of other antioxidants is an approach for prevention of myocardial injury. In addition to an antioxidant, vitamin E is involved in immune function, cell signaling, regulation of gene expression, and other metabolic processes. Several observational studies have associated lower rates of heart disease with higher vitamin E intakes (prevent oxidation of LDL and lipid peroxidation) (Cannon *et al.* 1991, Devaraj *et al.* 1996, Ricciarelli *et al.* 1998).

2.6 Green Tea

Green tea is proposed to be a dietary supplement in the prevention of cardiovascular diseases in which oxidative stress and proinflammation are the principal causes (Tipoe *et al*, 2007). GT because of its catechins reduced the risk of coronary heart disease by lowering plasma levels of cholesterol and triglyceride. Studies indicate that green tea catechins particularly (-)- epigallocatechin gallate, interfere with the emulsification, digestion and micellar solubilization of lipids, the critical steps involved in the intestinal absorption of dietary fat, cholesterol and other lipids (Koo and Noh, 2007).

2.7 Lycopene

Lycopene has been shown to be one of the most efficient singlet oxygen quencher and peroxyl radical scavengers among all the carotenoids and is about 100-fold more effective than α-tocopherol. A number of studies shown that lycopene can protect native LDL from oxidation and can suppress cholesterol synthesis. Lycopene effectively protects adrenaline induced myocardial infarction, adriamycin-induced cardiotoxicity, Doxorubicine induced cardiotoxicity and myocardial injury after ischemia and reperfusion. It also exerts an antiatherogenic effect by inhibiting the expression of inflammatory agents in hyperhomocysteninemic rats (Napolitano *et al*, 2007; Bansal *et al*, 2006).

2.8 *Lagenaria siceraria*

Lagenaria siceraria fruits are traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion strings, alternative purgative, cooling effects. Research suggested that *Lagenaria siceraria* fruit posses antihyperlipidemic (Ghule *et al*, 2006a), analgesic, anti-inflammatory (Ghule *et al*, 2006b), immunomodullatory, diuretics, antioxidant, cardioprotective (Fard *et al*, 2008), and hepatoprotective activity. The fruit of LS is not evaluated for its cardioprotective effect in isoproterenol induced myocardial injury yet.

2.9 Pomegranate

Pomegranate fruit have been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation in animals and increase plasma antioxidant capacity in elderly humans. Studies in rats and mice confirm the antioxidant properties of a pomegranate by-product (PBP) extract made from whole fruit minus the juice, showing a 19% reduction in oxidative stress in mouse peritoneal macrophages (MPM), a 42% percent decrease in cellular lipid peroxide content, and a 53% increase in reduced glutathione levels. The beneficial constituents in pomegranate are ellagic acid ellagitannins, punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones (Lansky *et al*, 2007).

2.7 Combination treatment

Antioxidants are uniquely different from one another and each may have the specific function in the body. However, they are also synergistic, and will work most effectively when they are used together. In the proper combination, they can perform a wide range of metabolic activities, free radical scavenging and preventative actions. Several *in vitro* and *in vivo* studies have reported that combination of vitamins with other antioxidants and a combination of hydrophilic and lipophilic antioxidants produces synergistic effects (Lo pez-Burillo *et al*, 2003; Jia *et al*, 1998; Pignatelli *et al*, 2000; Haramaki *et al*, 1998; Kagan *et al*, 1992; Brown *et al*, 2001; Yogeeta *et al*, 2006; Punithavathi and Prince, 2009; Pour *et al*, 2008).

In vitro study shows the synergistic effects of green tea polyphenol and vitamin E in miscelles, homogenous solutions and in human low density lipoproteins (Zhou, 2005). Studies also show the synergistic effects of lycopene with vitamin E in microzomal membranes and LDL oxidation (Palozza and Krinsky, 1993; Fuhrman *et al*, 2000). However, no systemic scientific evidence for the cardioprotective effect of combination of vitamin E and green tea, lycopene, LSFJ, PGFE *in vivo* has

been established till date. Hence the present study is designed to carry out effects of Vit.E alone and with GT, LYP, PGFE or LSFJ in myocardial infarction induced by isoproterenol.