2.1 **Global burden of ischemic heart disease and its prevalence in Indian population**

Ischemic heart disease (IHD) is a major cause of disease burden, ranked fourth globally and second in high and middle income countries (Murray and Lopez, 1996). India is experiencing an alarming increase in ischemic heart disease, which seems to be linked to changes in lifestyle and diet, rapid urbanization and possibly an underlying genetic component. According to the World Health Organization (WHO) estimates 60% of the world’s cardiac patients were reported in India by 2010 (Thomas et al., 2006). Over 80% of deaths and 85% of disability from cardiovascular disease (CVD) occur in low and middle income countries (Yusuf et al., 2001; Reddy, 2004; Leeder et al., 2004). The Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanka and Nepal) is home to 20% of the world’s population and may be one of the regions with the highest burden of IHD in the world. The huge burden of IHD in the Indian subcontinent is the consequence of the large population and the high prevalence of IHD risk factors. Moreover, the projected increase in deaths and disability from IHD is expected to follow closely an explosion in the prevalence of traditional risk factors. Deaths from IHD in India rose from 1.17 million in 1990 to 1.59 million in 2000 and reached to 2.03 million in 2010 (Ghaffar et al., 2004). In addition to the high rate of IHD mortality in the Indian subcontinent, IHD manifests almost 10 year earlier on an average in this region compared with the rest of the world (Ghaffar et al., 2004; Gupta 2004; Yusuf et al., 2004; Gupta, 2005).

In India, the IHD rate is expected to rise in parallel with the increase in life expectancy, secondary to increase in per capita income and declining infant mortality. The average life expectancy has increased from 41 years in the year 1951 to 1961, to 61.4 years in the year 1991 to 1996 and is projected to reach 72 years by 2030, which
could lead to large increases in IHD prevalence (Reddy et al., 1998). According to the Global Burden of Disease Study, a 55% rise would occur in DALY (disability-adjusted life year) loss attributable to IHD between 1990 and 2020 in the developing countries (Murray and Lopez, 1996), Figure 1.

![Figure 1 - Burden of ischemic heart disease (IHD), 1990-2020](image)

(IND, India; CH, China; SSA, Sub-Saharan Africa; MEC, Middle Eastern crescent; LAC, Latin America; EME, established market economies; FSE, former Socialist economies; OAI, other Asia and islands).


### 2.2 Myocardial ischemia and infarction-A paradigm for CVD

Myocardial ischemia and subsequent cardiomyocyte injury, even necrosis are the main pathological changes of cardiovascular disease. Myocardial ischemia is a state of myocardial impairment, which results from inadequate coronary perfusion of oxygenated blood relative to metabolic demands of the myocardium. The Greek ischo
means 'to hold back' and haima means 'blood'. Ischemia in the heart was first described and defined by the German pathologist Rudolf Virchow in 1858. It is characterized by an imbalance between myocardial oxygen supply and demand due to decrease of blood flow secondary to narrowing of the coronary artery (Virchow, 1858; David et al., 2007).

Myocardial ischemia therefore exists when the reduction of coronary flow is so severe that the supply of oxygen to the myocardium is inadequate for the oxygen demands of the myocardial tissue (Hearse et al., 1994). Lack of adequate oxygen supply to the mitochondria rapidly decreases the energy available to the cytoplasm. The rapid rundown of high energy phosphates, especially of creatine phosphate, stimulates glycolysis and glycogenolysis and glycolytic flux is promoted to a greater extent than its end products (pyruvate and NADH2) can enter the oxygen deprived mitochondria. Potassium also escapes from ischemic cells, in part due to activation and opening of the potassium channels that are normally inhibited by ATP. Oxidation of fatty acids is reduced and there is an accumulation of lipid metabolites, including intracellular free fatty acids, acyl CoA and acyl carnitine with adverse detergent properties. Moreover during ischemia, due to poor washout of metabolites lactic acid may accumulate resulting in decreased contractile activity, promotion of mitochondrial damage, decrease of action potential duration and inhibition of glycolysis at the level of glyceraldehydes 3-phosphate dehydrogenase (Hochachka et al., 1996). Thus ischemic heart disease involves an imbalance in the normal integrated function of the coronary vasculature and the myocardium. If blood flow to the myocardium is severely compromised or protracted, irreversible changes may occur in the heart.

Many theories have been advanced for such contractile failure. The two basic mechanisms most frequently advanced relate to either (i) the effect of poor oxygen
delivery or (ii) the accumulation of inhibitory metabolites. The first factor results in
depletion of high energy phosphate compounds, including ATP and creatine
phosphate. The second hypothesis is old and currently much favored is that, it is the
accumulation of products of ischemia that causes pump failure.

2.3 Catecholamines: Its role and participation in cardiac function

The sympathetic nervous system (SNS) regulates cardiac function through the
release of noradrenaline and subsequent activation of beta-adrenergic receptors (β-AR) and to some extent, alpha-adrenergic receptors (α-AR) (Rehsia and Dhalla, 2010). In response to stressors, an animal’s central nervous system triggers
physiological responses that ultimately result in activation of the hypothalamic
pituitary adrenocortical axis and/or the sympato-adreno medullary system which are
presumed to have adaptive values during periods of stress as they help in regaining
homeostasis (Moberg, 2000; Romero, 2004). The sympato-adrenal system consists
of the sympathetic nervous system with cholinergic preganglionic and adrenergic
postganglionic nerves and the adrenal medulla. The hormones of the sympathoadrenal
system, while not necessary for life are required for adaptation to acute and chronic
stress. The similar chemical structures of norepinephrine and epinephrine gives rise to
the family name of catecholamines. Catecholamine, such as epinephrine (adrenaline),
norepinephrine (noradrenaline) and dopamine are major elements in the response to
severe stress. Catecholamine plays an important role in the regulation of normal
cardiac function. A flow diagram depicting the effect of catecholamine cardiac
metabolism and function is presented in Figure 2.

Catecholamines are also known to produce a wide variety of direct and
indirect pharmacologic actions on cardiovascular hemodynamics and metabolism.
As a consequence of these complex effects, it has been difficult to determine whether
catecholamines exerts a direct toxic influence on the myocardium or whether myocardial cell damage is in some way secondary to other actions of catecholamines (Rona et al., 1959; Handforth, 1962; Rona et al., 1963 and 1973). Nevertheless, excessive release of catecholamines are responsible for the development of various cardiac dysfunctions viz. in cardiac remodelling following acute myocardial infarction and myocyte death in heart failure (Carelock et al., 2001; Goldspink et al., 2003; Piano et al., 2003).

![Diagram of the effects of catecholamines on myocardial metabolism and cardiac function](image)

**Figure 2- The effects of catecholamines on myocardial metabolism and cardiac function**
2.4 Isoproterenol-induced myocardial necrosis

Isoproterenol 4-[1-hydroxy-2-\{(propan-2-yl) amino\} ethyl] benzene-1, 2-diol is a synthetic catecholamine and a powerful non selective β-adrenergic agonist. ISP has been known to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle (Rona et al., 1959; Goyal et al., 2010; Shukla et al., 2012).

![Chemical structure of isoproterenol](image_url)

**Figure 3- Chemical structure of isoproterenol**

An illustration of ISP-induced myocardial infarction is presented in Figure 4 and 5. The model of ISP-induced myocardial ischemia is considered as one of the most widely used and well standardized experimental model to study the beneficial effects of several drugs and cardiac function (Dwivedi et al., 1988; Grimm et al., 1998) through an exaggerated pharmacological effect (O’Brien et al., 2006; York et al., 2007; Mikaelian et al., 2008; Schultze et al., 2008; Zhang et al., 2008; Clements
et al., 2010). It is a reliable, reproducible and well characterized model of cardiac hypertrophy associated with arrhythmias, myocyte loss and fibrosis with progression to heart failure (Szabo J, 1975).

The pathophysiological changes following ISP administration are comparable to those taking place in human myocardial ischemia/infarction (Wexler, 1978). Low concentrations of catecholamines exert positive inotropic action on the myocardium and thus are considered to be beneficial in regulating cardiac function. On the other hand, high concentrations of catecholamines over a prolonged period produce deleterious effects on the myocardium. It has been known for many years that epinephrine, norepinephrine and isoproterenol can causes cardiac hypertrophy and/or myocardial lesions (Szakkacs and Cannon, 1958; Rona et al., 1959). These myocardial lesions has been considered to represent catecholamine induced myocardial cell damage, catecholamine-induced myocardial infarction, or myocarditis. The LD$_{50}$ of isoproterenol in rats has been reported to be 680 mg/kg. Doses as low as 0.02 mg/kg produced microscopic focal necrotic lesions. The severity of myocardial damage has been closely related to the dosage of isoproterenol used and varied from local lesions affecting single cells to massive infarcts involving large portion of the left ventricle.

Isoproterenol-induced myocardial lesions are generally found to be localized in the apex as well as left ventricular subendocardium. Moreover these lesions are observed less frequently in the papillary muscle and right ventricle. Isoproterenol is also found to produce apical lesions and disseminated focal necrosis (Rona et al., 1959). Catecholamines are known to produce a wide variety of direct and indirect pharmacologic actions on cardiovascular haemodynamics and metabolism
Several mechanisms, such as cardiovascular haemodynamics and metabolic changes, alterations in the sarcolemmal permeability, formation of oxidation products of catecholamines and accumulation of catecholamine metabolites during the monoamine oxidase reaction has been suggested to explain the pathogenesis of catecholamine induced cardiac damage (Rosenblum et al., 1965; Maruffo, 1967; Bhagat et al., 1976; Boutet et al., 1976). Isoproterenol is known to increase cholesterol, triglyceride and free fatty acid in plasma and heart tissue (Farvin et al., 2006). Similarly, ISP significantly decreases levels of endogenous myocardial antioxidants CAT, SOD and GSH and increases the levels of cardiac markers (LDH, CPK, SGOT and Troponin I) along with increase in myocardial lipid peroxides (Ansari et al., 2006; Sachdeva et al., 2010; Parveen et al., 2011).

2.5 Mechanisms of catecholamine-induced myocardial ischemia

The exact mechanism of ISP-induced myocardial damage has not been clarified, but a mismatch of oxygen supply versus demand following coronary hypotension and myocardial hyperactivity may offer the best explanation for the complex morphological alterations. It has been assumed that myocardial cell necrosis following administration of catecholamine is due to defect in the supply of energy for the maintenance of essential cellular processes. Various theories have been proposed to suggest the cause of the energy deficiency and the nature of the irreversible step following decreased energy availability (Figure 2). The major hypothesis includes:

1. A relative cardiac hypoxemia caused by increased cardiac work and myocardial oxygen demands, aggravated by hypotension in isoproterenol-induced myocardial ischemia.
2. Coronary arterial vasoconstriction (spasm) causing endocardial ischemia.

3. Inadequate perfusion of the endocardium due to impaired venous drainage of the heart.

4. Hypoxia due to direct oxygen wasting effects of catecholamines or their oxidation products.

5. Interference with mitochondrial oxidative phosphorylation by free fatty acid.

6. Occurrence of intracellular Ca\(^{2+}\) overload as a result of massive calcium influx.

7. Formation of adrenochromes and their oxidation products including oxy radicals.

8. Potassium depletion and altered permeability of the myocardial cell membrane through elevation of plasma non esterified free fatty acids.


10. Promote direct tissue injury through oxygen derived free radical formation.

The primary disturbance of ISP-induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting increased cAMP formation; this in turn would have lead to the higher lipid accumulation in the myocardium. Increased lipolysis and peroxidation of endogenous lipids also play a major role in the cytotoxic action of isoproterenol (Buja, 1991). A considerable body of clinical and experimental evidence now exists suggesting the involvement of free radical mediated oxidative process in the pathogenesis of ISP-induced myocardial infarction (Rathore et al., 1998; Mohanty et al., 2004; Gauthaman et al., 2006; Farvin et al., 2006; Padmanabhan and Prince, 2006).
Figure 4- Effect of catecholamines on cytosolic calcium and energy state of myocytes
2.6 Manifestations due to isoproterenol administration

2.6.1. Reactive oxygen species (ROS): Generation and their cellular source during ischemia

ROS consisting of superoxide anion, hydrogen peroxide and hydroxyl radical have long been implicated as a major initiator of myocardial injury during ischemia/reperfusion on the basis of the following findings, (a) ROS are rapidly detected in the ischemic myocardium during the first minute after reperfusion (Chambers et al., 1985). (b) Experimental application of ROS equivalents causes alterations in the myocardium that are similar to those resulting from reperfusion (Mergner et al., 1991). (c) Treatment with antioxidant agents or up regulation of endogenous antioxidant enzymes, such as SOD and GSHPx in animals provides protection against ischemic injury (Jolly et al., 1984; Ambrosio et al., 1991).

Sources of ROS in Vascular Cells

Potential enzymatic cellular sources of reactive oxygen species (ROS) include neutrophils/monocytes, cardiomyocytes and endothelial cells, through enzymes such as the NAD (P) H oxidases, xanthine oxidase, cytochrome P450, nitric oxide synthase and mitochondria. Mitochondrial damage caused by ROS and reactive nitrogen species (RNS) is presented in Figure 6. Production of ROS in the heart can be induced by a number of cytokines and growth factors, such as angiotensin II, platelet derived growth factor and tumor necrosis factor-α through activation of NAD (P) H oxidases. Uncoupled mitochondria could also be a major source of ROS in heart failure (Ide T et al., 1999). The neutrophil enzyme, myeloperoxidase is increased in heart failure, suggesting these cells to be another source of ROS (Tang et al., 2006; Ng LL et al., 2006). Although many of these sources could potentially produce ROS that inactivate NO. Generally cardiovascular disease is characterized by ROS overproduction whereas the formation of the major free radicals and peroxynitrite can decrease or increase depending on the nature of heart injury.
The oxygen radical hypothesis is of particular importance because it can potentially explain each of the other mechanisms of ischemic injury. It has been demonstrated that free radicals causes cellular calcium loading with inhibition of sarcoplasmic reticulum calcium ATPase and inhibition of the sodium potassium ATPase leading to the sodium mediated calcium gain (Hess et al., 1981). Oxygen radicals also cause lipid peroxidation that can result in cell membrane breakdown, producing cell swelling and edema. It has been suggested that oxygen radicals result in the chemotaxis of neutrophils that in turn can lead to white cell plugging of capillaries and microvascular compression. In addition white cells activated and act as potent source of further oxygen radical generation.

Figure 5- Schematic diagram of ISP-induced cardiotoxicity: role of superoxide, NO and peroxynitrite
(Source: modified from Mukhopadhyay et al., Am J Physiol Heart Circ Physiol, 2009)
2.6.2 Oxidative stress in myocardial infarction

Oxidative stress is a state in which excess formation of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) saturate the natural antioxidant defence mechanisms (Turko et al., 2001; Maritim et al., 2003). The cardiovascular system is continuously exposed to both reactive oxygen and nitrogen species (Dhalla et al., 2000). Oxygen, although essential for tissue survival can be injurious during reperfusion of previous ischemic myocardium.

Compelling evidence for role of free radical injury comes from a number of sources. By virtue of their unpaired electron, free radicals are typically unstable and highly reactive and short lived. Oxygen derived free radicals and oxidants (reactive oxygen species) are formed continuously in small amounts during the normal metabolism of cells and are normally inactivated by endogenous scavenging mechanisms. Free radicals are generated by one electron reduction or oxidation of molecules creating an unpaired electron. In normal mitochondrial oxidative phosphorylation, $O_2$ is reduced by four electrons to form $H_2O$. The energy derived from this reduction of $O_2$ serves to meet the energy demands of the cell. Paradoxically, it is also the actual process of oxygen reduction that leads to the formation of oxygen free radicals. Incomplete reduction of $O_2$ leads to the generation of peroxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical (OH). $O_2^-$ is unstable with a half life of millisecond at neutral $pH$, and in aqueous solution it spontaneously reacts or dismutates to yield $H_2O_2$ and $O_2$. The -OH is an extremely reactive and short lived free radical produced in biological systems. ROS injured cells by causing peroxidation of membrane lipid, denaturation of proteins including
enzymes and ion channels and strand breaks in DNA. Lipid peroxidation triggers loss of membrane integrity, necrosis and cell death (Park and Lucchesi, 1999). Oxidative damage by ROS has been documented in a number of experimental studies from subcellular and cellular to in vitro and in vivo models and in humans (Hearse, 1998; Ciconi et al., 2003; Banyopadhyay et al., 2004). The oxygen paradox hypothesis was first introduced by Hearse, 1998 where they documented that re-oxygenation of the hypoxic rat heart resulted in significant damage rather than improvement. Later on Lucas et al., 1980; Stewart et al., 1983 and Jolly et al., 1984 substantiated this concept in the canine and rabbit model of global ischemia using known oxygen radical scavengers. In these studies, the ability of oxygen radical scavengers to improve mechanical, sarcoplasmic reticulum and mitochondrial function suggested that oxygen free radicals participate in experimental ischemic injury (Zweier et al., 1988; Zweier et al., 1989). It has been established that oxygen free radical mediated injury is a central mechanism of myocardial ischemic injury. A number of mechanisms have been proposed to cause oxygen radical generation in reperfused myocardium (Zweier et al., 1988; Zweier et al., 1989; Hansen, 1995; Flaherty et al., 1997; Gross et al., 1999). These include: the enzyme xanthine oxidase, mitochondrial oxidation, catecholamine oxidation, P450 mediated oxidation, activation of leukocyte NADPH oxidase, iron release and redox cycling. A number of changes occur in ischemic tissues that predispose the tissue to subsequent oxidative reperfusion injury. It has been demonstrated that enzyme xanthine dehydrogenase is converted to xanthine oxidase, a potent generator of O2⁻ and hydrogen peroxide (Chambers et al., 1985).
2.6.3. Hemodynamic changes

Under a wide variety of stressful conditions, the circulating levels of catecholamines are markedly elevated. Occurrence of coronary spasm and arrhythmias has been well recognized in ischemic injury. In fact coronary spasm is considered to result in arrhythmia, myocardial ischemia and myocardial cell damage as a result of catecholamines. The hypothesis of catecholamine-induced cardiac necrosis is closely related to coronary insufficiency of hemodynamic origin of a relative ischemia resulting from coronary vascular changes. It was found that isoproterenol changed the distribution of uniform coronary flow in endomyocardium (Handforth, 1962). Dilation of AV shunts might be responsible for the endocardial ischemia because coronary flow is usually increased with isoproterenol. It has been
believed that these results were caused by spasm of the coronary vessels. Changes in peripheral resistance due to catecholamines were also important because it was possible to reproduce essentially similar pathologic changes by surgical occlusion of the efferent vessels. Impairment of venous drainage via venospasm largely accounts for the adverse effects of sympatho mimetic amines. Blood flow to the left ventricular subendocardial muscle has been suggested to be compromised during systole and to occur mainly during diastole because intra myocardial compressive force is greatest in this region (Figure 7).

Figure 7 - Effects of isoproterenol on hemodynamic and ventricular function
2.6.4 Cardiac markers

When heart muscle fibers die, intracellular enzymes are released from the cells into surrounding tissue and appear in the circulating blood (Puleo et al., 1994; Pentilla et al., 2002). The diagnostic techniques for detecting myocardial damage have changed greatly since the decline in cardiovascular disease mortality rates began in the late 1960. The enzymes of greatest diagnostic value for myocardial infarction are creatine phophokinase myocardial band (CPK-MB), lactate dehydrogenase (LDH), cardiac troponin-I (cTnI) and serum oxaloacitate glutamate transaminase (SGOT) (Panteghini, 1988; Puleo et al., 1994; Christenson et al., 1998; Boccara et al., 2000; Ooi et al., 2000). This diagnostic drift has been driven largely by the availability of new and more sensitive cardiac enzymes or biomarkers. Creatine kinase (CK) is an enzyme that catalyses the transfer of phosphate from creatine phosphate to ADP to form ATP. There are 3 major CK isoenzymes identified (MM, MB, and BB) that exhibit some degree of tissue specificity. CK-MM is the principal form in skeletal muscle, both CK-MM and CK-MB are present in myocardium and CK-BB is the predominant form in brain and kidney. Heart tissue has virtually specific isoenzyme, which is a hybrid of both subunits and is termed CPK-MB. CPK-MB is found almost exclusively in heart muscle and it is indicative of an acute MI, when found in serum (Puleo et al., 1990). During acute myocardial infarction, CPK-MB appears in 4 to 8 h, reach peak activity at 18 to 24h and may last for another 2 days. CPK-MB has been found to be very specific and very sensitive for an acute MI. Since CPK-MB may be released in conditions involving reversible myocardial injury, such as ischemia, congestive heart failure or tachyarrhythmia, other diagnostic criteria besides CPK-MB elevation alone would appear warrantee to establish the diagnosis of an acute MI reliably.

The enzyme serum oxaloacitate glutamate transaminase (SGOT) is normally present inside the cells. When the cell is damaged these enzymes leak out and when a large number of cells are damaged their level in the blood will rise. It has been shown
to rise clinically in myocardial infarction. SGOT is found in heart muscle, liver, skeletal muscle, kidneys and the pancreas. This enzyme shows an elevation 8-12 hrs after infarction and peak levels are reached 24-48 hrs after the MI. This enzyme is not particularly indicative of MI. High levels of SGOT may be due to trauma in the skeletal muscles, in liver disease, pancreatitis and others. Use of SGOT with other enzyme results in more definite diagnosis the MI.

Serum cardiac troponins (cTnI and cTnT) are the earliest appearing biochemical markers during myocardial damage (Christenson et al., 1998; Boccara et al., 2000; Ooi et al., 2000). cTnI and cTnT are specific, sensitive and robust biomarkers of myocardial damage that are released into the serum soon following tissue pathogenesis and reflect the extent of irreversible myocardial cell injury caused by both natural and drug-induced diseases in humans and common laboratory species. The troponins present a potential bridge between nonclinical and clinical studies in that they appear to be useful biomarkers for assessing drug-induced cardiac injury in both humans and animals.

Elevated serum cTnI or cTnT may reflect a direct effect of the drug on the myocardium or may be secondary to a drug effect on some other organ system that subsequently leads to myocardial damage. It is unclear to what extent troponin would be released by cells that die by apoptosis during ischemic injury or other types of cardio toxicity. Depending upon the amount of secondary necrosis following apoptosis, there could be apoptotic myocardial injury that does not lead to release of troponin into the serum. Within the context of a given pathogenic process, the magnitude of increase in serum cTnI or cTnT correlates with the extent of myocardial cell injury. There may exist thresholds for troponin release below which the myocardial injury is fully compensated at the organ level and there are no associated functional deficits. Immunoreactive cTnI is localized exclusively in the heart and is not known to be expressed in skeletal muscle or any other tissue, even in diseased
states. While cTnT isoforms are expressed in injured skeletal muscle, they are not detected by the current diagnostic assays. The unique aspect of cTnI is being 100% tissue specificity for the heart (Bodor et al., 1995) making it an excellent marker, biochemical and immunohistochemical tool for detecting myocardial injury. The continuing cellular release and clearance of cTnI account for its excellent diagnostic sensitivity and quantitative measure of cardiac injury in humans (Adams et al., 1993; Babuin and Jaffe, 2005) and veterinary species (O’Brien et al., 2006). Concentrations in serum are increased as a result of direct myocardial injury, myocardial ischemia, ventricular strain caused by disease (Fromm, 2007) or drug-induced toxicity (Babuin and Jaffe, 2005; Adamcova et al., 2007). Troponin levels may remain elevated for 7-10 days after an episode of myocardial infarction (Gupta and deLemos, 2007).

**Table 1 - Biomarkers used or proposed for use in the clinical diagnosis of acute MI**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Molecular weight (daltons)</th>
<th>Initial elevation (hrs)</th>
<th>Peak elevation (days)</th>
<th>Time to return to baseline (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>86,000</td>
<td>3-12</td>
<td>1</td>
<td>2-3</td>
</tr>
<tr>
<td>cTnI</td>
<td>22,000</td>
<td>3-12</td>
<td>1</td>
<td>5-10</td>
</tr>
<tr>
<td>cTnT</td>
<td>37,000</td>
<td>3-12</td>
<td>0.5</td>
<td>5-14</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17,800</td>
<td>1-4</td>
<td>0.5-0.6</td>
<td>1</td>
</tr>
<tr>
<td>LDH</td>
<td>135,000</td>
<td>10</td>
<td>1-2</td>
<td>10-14</td>
</tr>
<tr>
<td>SGOT</td>
<td>92,000</td>
<td>6-8</td>
<td>24</td>
<td>4-6</td>
</tr>
<tr>
<td>hFABP</td>
<td>14,000-15,000</td>
<td>1.5</td>
<td>0.4-0.9</td>
<td>1</td>
</tr>
</tbody>
</table>

2.6.5 Pro-inflammatory cytokines

The term ‘proinflammatory cytokines’ includes many cytokines characterized as being inducible and belonging to different families, including IL-1, IL-6, TNF-α and CRP (Beutler and Cerami, 1989; Kishimoto et al., 1992; Smith et al., 1994; Dinarello, 1998). Pro-inflammatory cytokines are not constitutively expressed in the normal heart (Kapadia et al., 1995; Kapadia et al., 1997) but their expression can be triggered after myocardial infarction (Irwin et al., 1999; Deten et al., 2002) by tissue injury (Mann DL, 2003) and mechanical stress (Kapadia et al., 1997). Early after MI, cytokine production participates in the recruitment of inflammatory cells, contributing to the natural process of myocardial healing. Acute treatments that inhibit cytokine production after MI can affect this beneficial early response and therefore aggravate the prognosis of MI. From the literature it appears that after an initial raise in the infarcted region of the myocardium, the cytokine level decreases to return to basal values after 24-48 h (Gurantz et al., 2005).

Interleukin-6 (IL-6)

IL-6, a 185 amino acid polypeptide is a predominantly pro-inflammatory cytokine. IL-6 is produced by a variety of leukocytes and exerts its biological activity by binding the IL-6R, which heterodimerizes with its gp130 signaling component. It is a non antibody protein and intercellular mediator. It acts as the primary driver of hepatic CRP synthesis. This marker is produced by a variety of cells in the body. Plasma concentrations of IL-6 reflect the intensity of plaque vulnerability to rupture and restenosis following percutaneous coronary intervention (Hirano, 1998; Wen et al., 2012). IL-6 is involved in the pathogenesis of the acute coronary syndrome and has the following effects: stimulates the linear production of fibrinogen and CRP, stimulates the macrophage to produce tissue factor and matrix metalloproteinase, platelet aggregation, adhesion molecules, tumor necrosis factor-α and vascular smooth
muscle cell proliferation. IL-6 has some undeniably pro-inflammatory features, for example it induces fever. IL-6 can have anti-inflammatory effects (Somers et al., 1997). The anti-inflammatory feature such as the capacity to partly down regulate TNF-α production. IL-6 also has major effects on the immune system, activating B cells to produce immunoglobulin and T cells to up regulate receptors for IL-2 (Kishimoto et al., 1992). Elevation of circulating IL-6 is a strong and independent marker of mortality in ischemic heart disease.

**C-reactive protein (CRP)**

CRP is an acute-phase protein produced by the liver. CRP is elevated during tissue injury, infection or inflammation. CRP is prothrombotic, which promotes tissue factor production, macrophage uptake of low density lipoprotein, vascular cell adhesion molecule expression and induces monocyte chemo attractant protein-1 (mcp-1). Elevated level of CRP is associated with an increased risk of recurrent events in the IHD. Circulating CRP values correlate closely with other markers of inflammation, some of which show similar, albeit generally less significant, predictive associations with coronary events (Danesh et al., 1998; Danesh et al., 1999). The attention focused on CRP reflects in part the fact that it is an exceptionally stable analyte in serum or plasma. The immunoassays for it are robust, well standardized, reproducible and readily available. Furthermore, the intrinsic biological properties of CRP as an acute phase reactant are, as explained above especially favorable for its use as a sensitive quantitative systemic readout of the acute-phase response.

Tissue necrosis is a potent acute-phase stimulus during myocardial infarction that shows major CRP response, the magnitude of which reflects the extent of myocardial necrosis (de Beer et al., 1982). Furthermore, the peak CRP values are detectable after 48 hrs of the onset of MI (de Beer et al., 1982; Pietila et al., 1996;
Importantly, CRP is co-deposited with activated complement within all acute myocardial infarcts (Kushner et al., 1963; Lagrand et al., 1997). The compelling experimental evidence now suggests that the CRP response not only reflects tissue damage, but may also contribute significantly to the severity of ischemic myocardial injury (Griselli et al., 1999).

**Tumor necrosis factor alpha** (TNF-α)

TNF-α exists as a homotrimer, consisting of three 17 kDa subunits, with a molecular mass of ~51 kDa (Arakawa et al., 1987; Utsumi et al., 1992). TNF-α is a pro-inflammatory, pro-apoptotic cytokine that regulates the activity of neutrophils, eosinophils and T & B lymphocytes. TNF-α has potent negative inotropic properties and it modulates the properties of the vascular endothelium (Balkwill, 1989). Elevated level of serum TNF-α is associated with depressed myocardial function (Suffredini et al., 1989). TNF-α is more predictive of future cardiovascular events as compared to CRP (Cesari et al., 2003). TNF-α is synthesized as a 26 kDa propeptide (pro-TNF-α) in the cytosol, which is then cleaved to a 17 kDa active form by TNF-α-converting enzyme (TACE). This cleavage occurs as pro-TNF-α passes through the cell membrane.

The activated form of TNF-α binds to the receptors on the cell membrane surface and triggers alterations in cytosolic protein synthesis and activation of different kinases (Torre-Amione et al., 1995; Sack M, 2002). TNF-α is a pleiotropic cytokine produced by a variety of cells, including macrophages, endothelial cells, and smooth muscle cells. TNF-α has a central role in the amplification of the inflammatory cascade. However, the plasma half-life of TNF-α is short, a factor that limits its potential clinical utility as a screening tool. TNF-α, contribute to the pathogenesis and progression of heart failure and also directly correlate with the
severity of the disease (Stumpf et al., 2003; Anker et al., 2004). Elevation in TNF-α level is linked with an increased incidence of congestive heart failure after myocardial infarction (Vasan et al., 2003).

It has been shown that the failing myocardium secretes TNF-α and both cardiomyocytes and non cardiomyocytes from the infarcted heart show elevated levels of TNF-α gene expression and TNF-α protein (Torre-Amione et al., 1996; Kapadia et al., 1997; Ono et al., 1998; Yue et al., 1998). Moreover, TNF-α can induce cardiomyocyte apoptosis and destabilization of atherosclerotic plaques (Libby et al., 1995; Pulkki et al., 1997). It was found that TNF-α is expressed within the myocardium of rats (Herskowitz et al., 1995) and dogs (Gwechenberger et al., 1999) in response to ischemic injury (Mann DL, 1996). TNF-α has dual effects in apoptosis. It provokes the expression of several anti-apoptotic factors in a variety of mammalian cell types, although it can also induce apoptosis in mouse fibroblasts (Beg et al., 1996).

2.6.6 Histopathological changes during MI

Histopathological changes in catecholamine-induced cardiac damage are generally characterized by degeneration and necrosis of myocardial fiber, accumulation of inflammatory cells such as leukocytes, interstitial edema, lipid droplet or fat deposition and endocardial hemorrhage. Subendocardial layers being subject to greater mechanical stress, require an increased oxygen uptake, which accounts for the lower tissue oxygen tension and higher rate of oxidative metabolism in the subendocardium. These metabolic factors in addition to blood flow factors, may account for the increased vulnerability of the subendocardium to ischemic damage with increased risk of necrosis. Histological changes following isoproterenol
challenge in rats are edema, inflammatory cell infiltration and extensive myonecrosis. Interstitial edema, produced by isoproterenol is usually associated with subendocardial and subepicardial haemorrhages, which is characteristically present in damaged areas of the myocardium (Chappel et al., 1959; Rona et al., 1959; Rona et al., 1963; Rosenblum et al., 1965; Maruffo, 1967; Ferrans et al., 1969; Lehr, 1972).

**Proposed pathogenesis of post-ischemic myocardial dysfunction**

![Diagram](image)

**Figure 8- Illustration of the proposed pathogenesis of post-ischemic myocardial dysfunction**

(Source: modified from Bolli R and Marba E Physiol Rev, 1999)
The pathogenesis presented in Figure 8 integrates and reconciles different mechanisms into a unifying hypothesis. Transient reversible ischemia followed by reperfusion could result in increased production of superoxide radicals ($O_2^-$) through several mechanisms, including (i) increased activity of xanthine oxidase (ii) activation of neutrophils (iii) activation of arachidonate cascade (iv) accumulation of reducing equivalents during oxygen deprivation (v) derangement of the mitochondrial electron transport system resulting in increased univalent reduction of oxygen and (vi) autooxidation of catecholamines and other substances. Superoxide dismutase (SOD) convert $O_2$ to hydrogen peroxide ($H_2O_2$) in the presence of catalytic iron, $O_2^-$ and $H_2O$ interact in a Haber-Weiss reaction to generate the hydroxyl radical ($OH$). $H_2O_2$ can also generate $-OH$ in the absence of $O_2^-$ through Fenton reaction provided that other substances (such as ascorbate) reduce $Fe^{3+}$ to $Fe^{2+}$. $O_2^-$ and $-OH$ attack proteins and polyunsaturated fatty acids, causing enzyme inactivation and lipid peroxidation, respectively.

In the setting of reversible ischemia, the intensity of this damage is not sufficient to cause cell death, but is sufficient to produce dysfunction of key cellular organelles. Postulated targets of the free radical damage include (i) the sarcolemma, with consequent loss of selective permeability, impairment of calcium stimulated ATPase activity and calcium transport out of the cell and impairment of the $Na^+ K^+$ ATPase activity. The net result of these perturbations would be increased transsarcolemmal calcium influx and cellular calcium overload. (ii) The sarcoplasmic reticulum with consequent impairment of calcium stimulated ATPase activity and calcium transport. This would result in impaired calcium homeostasis: specifically, decreased calcium sequestration (which would contribute to increase free cytosolic calcium) and decreased calcium release during systole (which would cause excitation-contraction uncoupling). (iii) Possibly other structures, such as the extracellular collagen matrix (with consequent loss of mechanical coupling) or the contractile
proteins (with consequent decreased responsiveness to calcium). At the same time, reversible ischemia/reperfusion could cause cellular Na\(^+\) overload because of (i) inhibition of sarcolemmal Na\(^+\) K\(^+\) ATPase and (ii) acidosis and Na\(^+\)-H\(^+\) exchange. This could further exaggerate calcium overload via increased Na\(^+\)-Ca\(^{2+}\) exchange. An increase in free cytosolic calcium would activate protein kinases, phospholipases and other degenerative enzymes, further exacerbate the injury to the aforementioned key subcellular structures (sarcolemma, sarcoplasmic reticulum and contractile proteins). Thus, calcium overload could serve to amplify the damage initiated by oxygen radicals. In addition, calcium overload impair contractile performance and contribute to mechanical dysfunction. It is also possible that the increase in free cytosolic calcium could increase oxyradical production by promoting the conversion of xanthine dehydrogenase to xanthine oxidase. The ultimate consequence of this complex series of perturbations is a reversible depression of contractility.

2.6.7 Apoptosis and necrosis: The spectrum of cell death during myocardial ischemia

Apoptosis has long been identified as an evolutionarily conserved process of active cell elimination during development. Its phenotypic features include DNA fragmentation and chromatin condensation, cell shrinkage and formation of apoptotic bodies, which are cleared by phagocytosis without initiating a systemic inflammatory response. The execution of apoptosis requires novel gene expression and protein synthesis (Lockshin, 1969; Webster and Gross, 1970; Marovitz et al., 1976). Apoptosis has evolved as an intricate and critical mechanism for balancing cell proliferation and for the active remodeling of tissues during development. The identification of apoptosis under pathological settings dates back to the 1960, when John Kerr was studying ischemic liver damage. He observed a novel cell death phenotype that was morphologically distinct from classical necrosis. Since Kerr et al
in 1972 coined the term ‘apoptosis’ for a morphologically distinct mode of cell death, this concept of cell suicide has gained increasing interest in cytology and pathology. There is two distinct type of cell death in myocardium viz. necrosis or apoptosis. The common view on how cardiomyocytes die during or after myocardial infarction has altered in recent years. For a long time necrosis was regarded as the sole cause of cell death in myocardial infarction. Now, recent studies indicate that apoptosis also plays a role in the process of tissue damage subsequent to myocardial infarction. Although both necrosis and apoptosis result in the death of the cell, they differ in several morphological and cellular regulatory features. Necrosis is characterised by the rapid loss of cellular homeostasis, rapid swelling as a result of the accumulation of water and electrolytes, early plasma membrane rupture and the disruption of cellular organelles. Due to the membrane rupture and subsequent leakage of a broad array of cellular material, necrosis induces an inflammatory response (Kerr et al., 1972; Kerr et al., 1994; Majno and Joris, 1995). Apoptosis or programmed cell death is, unlike necrosis, a highly regulated and energy requiring process. Apoptosis is characterised by shrinkage of the cell and the nucleus. The nuclear chromatin is condensed into sharply delineated masses and eventually breaks up. The cell is then detached from the surrounding tissue. At this stage extensions bud out from its membrane, which eventually seals off to form membrane enclosed vesicles, called apoptotic bodies containing condensed cellular organelles and nuclear fragments. These apoptotic bodies are either rapidly phagocytosed by neighbouring cells or undergo degradation, which resembles necrosis in a process called secondary necrosis. However, apoptosis is generally considered not to trigger an inflammatory response (Saraste and Pulkki, 2000).

Accumulating evidence suggests that programmed cell death or apoptosis plays an important role in myocardial infarction. The occurrence of programmed cell death has clearly been shown in several clinical and experimental settings involving
myocardial injury. Although several studies have reported the appearance of apoptosis in the ischemic myocardium after a prolonged coronary occlusion without reperfusion. The fact that apoptosis plays a role in the tissue damage seen after myocardial infarction has pathological and therapeutic implications. Apoptosis is a highly regulated process, a better understanding of the circumstances that specifically trigger apoptosis during and after myocardial infarction and the cellular mechanisms that control apoptosis, could lead to therapeutic strategies to limit the amount of tissue damage in patients with myocardial infarction.

A considerable number of experimental studies and clinical observations has increasingly shown that myocardial apoptosis in addition to necrosis is primarily a reperfusion triggered phenomenon (Buja and Entman, 1998; Ohno et al., 1998; Dumont et al., 2000; Nadal et al., 2003). Although myocardial infarction was long considered to be characterized by non apoptotic (necrotic) cell death due to the breakdown of cellular energy metabolism. There is growing evidence that myocyte loss during the acute stage of myocardial infarction involves both apoptotic and nonapoptotic cell death. Observation in animal models of myocardial infarction suggest that apoptosis may contribute substantially to cell death even within the central infarct area with 5% to 33% of the cardiomyocytes staining positive for DNA fragmentation (Kajstura et al., 1996; Fliss and Gattinger, 1996; Bialik et al., 1997; Yaoita et al., 1998). However, at present the relative importance of apoptotic and non apoptotic cell death in both the acute and chronic phases of myocardial infarction is not known. Initial evidence for the potential pathophysiological significance of apoptosis has been reported in a rat model of myocardial infarction (Yaoita et al., 1998).

2.6.7.1 Regulation of apoptosis

Present concept of myocardial injury attributes the major portion of cell loss during cardiac ischemia to primary necrosis. However based on the observation that
apoptosis can be early and predominant from cell death in infarcted myocardium, the possibility of apoptosis as inducer of secondary necrosis has already been raised. Thus investigating the role of apoptosis in myocardial infarction as well as documenting its underlying mechanism may lead to new therapeutic strategies to prevent serious cell loss following ischemic injury. Further the effect of herbal drug on the cardiomyocyte apoptosis in the surviving myocardium after infarction has been demonstrated (Mohanty et al., 2006; Nandave et al., 2007).

Both caspase dependent and caspase independent apoptosis are regulated by the B-cell lymphoma-2 (Bcl-2) family of proteins, which include both pro-death and pro-survival members (Graham et al., 2000). Bcl-2 family proteins regulate the permeability of the mitochondrial outer membrane and permeability transition pore formation (Harris and Thompson, 2000). They contain highly conserved Bcl-2 homology domains (BH1-4) essential for homocomplex and heterocomplex formation (Graham et al., 2000). Complexes formed between proteins containing BH-3 domains such as Bax, truncated Bid, and Bad can facilitate the release of cytochrome c from mitochondria (Graham et al., 2000). Up regulation of Bax with subsequent mitochondrial translocation can be induced by the tumor suppressor p53, which is increased in injured regions after ischemic injury in rats (Bilsland and Harper, 2002; Concha and Abdel-Meguid, 2002). The anti-apoptotic members Bcl-2, Bcl-xL, and Mcl-1L prevent the release of mitochondrial proteins, including cytochrome C (Rosse et al., 1998), endonuclease G (Li et al., 2001) and AIF (Susin et al., 1999) by inhibiting the pore forming function of BH-3 domain containing Bcl-2 proteins (Antonsson et al., 1997). Existence of crosstalk between the extrinsic and intrinsic cell death pathways by means of Bid (BH3 domain protein) protein has been studied (Li et al., 1998). The anti-apoptotic gene Bcl-2 and its protein product are up regulated after antioxidant pre-treatment in rats and cells expressing Bcl-2 protein seem morphologically normal.
The proto oncogene Bcl-2 acts by promoting cell survival in contrast to other known oncogene viz. Src-kinase and Ras. Bcl-2 and Bcl-XL are known to counteract the pro apoptotic action of Bax (Kroemer et al., 1998). Some studies indicated that members of the Bcl-2 family may be pro apoptotic as well as may be involved in malignant growth. Subsequent studies revealed that Bcl-2 inhibited apoptosis induced by cytotoxic stimuli, chemotherapeutic drugs, or ionizing radiation. Hence, Bcl-2 family members constitute an important checkpoint of apoptosis for intracellular death pathways (Figure 9).

Figure 9- Suggested mechanisms to mediate the anti-apoptotic effect of Bcl-2
2.6.7.2 Antiapoptotic approaches

Four principal mechanisms for the Bcl-2–mediated anti-apoptotic effect have been proposed

1) Direct antioxidant effect.

2) Inhibition of the release of proapoptotic mitochondrial proteins.

3) Sequestration and/or modulation of the proapoptotic CED-4 protein and its mammalian homologue.

4) Inhibition of a direct cytotoxic effect of the proapoptotic regulators Bax and Bak.

The generation of ROS was initially suggested to be a common final pathway of apoptosis that could be abrogated by the antioxidant activity of Bcl-2 (Hockenbery, 1993).

Bax, a 21 KDa protein is the first pro apoptotic Bcl-2 family member, when over expressed, accelerates apoptosis. It also counters the death repressor activity of Bcl-2 (Oltvai et al., 1993). Bax plays a crucial role in the induction phase of apoptosis. In contrast to Bcl-2, Bax has a low basal expression in human hearts without cardiac disease (Misao et al., 1996). When transferred with Bcl-2, the death inducing effect of Bax was neutralized by heterodimerization with Bcl-2. When excess Bax/Bcl-2 heterodimers are formed, cells are protected. Whereas in hearts with predominantly Bax, the Bax homodimers render the myocytes susceptible to apoptosis (Lixin et al., 1998). The ability of Bax to block apoptosis is critically dependent on the ratio of Bcl-2 to Bax. Bcl-2 and Bcl-X<sub>L</sub> localize to other mitochondrial membrane, to other nuclear envelope and to the endoplasmic reticulum.

In the normal state of a cell, Bax is rather located in the cytosol. After an apoptotic stimulus, Bax translocates to the mitochondria and inserts in to the
mitochondrial membrane. Bax and Bak influence basic mitochondrial mechanisms required for the induction of apoptosis. Bcl-2 proteins do not regulate cell death extensively by the relative expression level of Bcl-2 related death agonists and antagonists. Homo and heterodimers of Bcl-2 homologs mainly locate to the mitochondrial outer membrane facing the cytosol, where they can interact with cytosolic effectors such as signal transduction molecules.

Apoptotic signalling induces apoptosis primarily through three types of complex pathways. They include (i) cytokine/Fas receptor driven pathway (ii) mitochondrial driven pathway and (iii) endoplasmic reticulum/Ca2+ driven pathway (Hengartner, 2000). Among them, mitochondrial mediated pathway, including the Bcl-2 family is the best characterized and believed to be critical in regulating apoptosis (Liu et al., 1998; Hengartner, 2000; Ruetten et al., 2001; Centurione et al., 2003). Indeed, progressive impairment of mitochondrial function is a hallmark and has been linked with apoptosis (Van Remmen and Richardson, 2001; Hagen et al., 2002; Pollack et al., 2002), Figure 10.
Figure 10- Overview of the signal transduction pathway of ISP-induced myocardial apoptosis.

In this figure, inducing or activating effects are depicted by an arrow and inhibitory effects are depicted by a blocked line (Source: Krijnen et al., J Clin Pathol, 2002)
2.7 Animal models for myocardial ischemia

Animal models that mimic cardiovascular diseases are indispensable tools for understanding the mechanisms underlying the diseases at the cellular and molecular level. Ischemic heart disease models have been developed in many species, including large animals such as swines and dogs (Murasato et al., 1997; Yang et al., 2006) as well as, small animals such as rats and mice (Imanishi et al., 2008; Lin et al., 2008; Das et al., 2009). Small animal models are more applicable to research work compared to large animal models due to their inexpensiveness, convenience in handling and the vast amount of scientific literature available (Cohen et al., 1994). MI in animal models can be mainly achieved by two methods. The first is to fully block or partially narrow the coronary artery, which often leads to acute ischemia. This can be achieved by a surgical procedure or by drug intervention. The other method is to induce atherosclerosis in coronary arteries, which would more closely mimic the disease progression in humans.

Drug-induced myocardial ischemia is a convenient procedure, since it does not require complicated surgery. Isoproterenol, adriamycin and ergonovine have been often used to induce MI. Studies related to isoproterenol intervention had been reported (Rona et al., 1959; Handforth, 1962; Rona et al., 1963 and 1973; Singal et al., 1982; Arnolda et al., 1985; Chagoya et al., 1997; Arteaga et al., 2002). Drug induced ischemia can be easily achieved. Since it increases myocardial oxygen consumption or induces coronary artery spasm to reduce blood flow; however, drug safety and the difficulty in accurately positioning the infarct region make this model rarely used in clinical research. To screen the drugs for their cardioprotective activity and to understand the pathophysiology of IHD a wide varieties of experimental animal models of myocardial infarction have generally used. They can be used either acute...
occlusion by ligation of coronary arteries or massive injections of catecholamines. The farmer procedure produces transmural myocardial infarction while the later causes subendocardial infarction. The development of regional ischemia in the anesthetized animal has been the model of choice for many years (Bhindi et al., 2006).

2.8 Antioxidants: Therapeutic and Preventive Potential

Due to a continuous generation of partially reduced forms of oxygen (PRFO) during cardiac cell metabolism, a number of protective enzymatic as well as non enzymatic antioxidants have evolved. They constitute an antioxidant reserve (Singal and Kirshenbaum, 1990) that act to limit the tissue concentration of these highly reactive species. A proper balance between the generation of PRFO and the antioxidant defense system is critical for the maintenance of a normal myocardial cell structure and function.

Antioxidants are substances that reduce oxidative damage in cells caused by free radicals. Several studies appear to find depletion of myocardial non enzymatic antioxidants with either a decrease or increase or no change in activities of myocardial enzymatic antioxidants. Ferrari et al., 1998 were the first to report the formation and release of oxidized glutathione (GSSG) in the coronary sinus following human myocardial ischemia reperfusion in patients. It has been consistently documented that oxygen derived radicals are generated during the post ischemic reperfusion period and directly contribute to post ischemic ventricular dysfunction and cell death (Reimer and Ldekker, 1987; Zweier et al., 1989; Ambrosio et al., 1999). Several studies demonstrated the protective role of antioxidants against oxygen derived radical injury (Burton et al., 1984; Ambrosio et al., 1991; Dhall et al., 2000; Bandyopadhyaya et al., 2004).
Hence search for the source of oxygen derived radicals, as well as interventional approaches against these damaging species are continued. Thrombolytic and antithrombotic drug therapy accounts for the success in treatment of patients with myocardial infarction. However, a novel drug yet to needed as an explicitly cardioprotective agent (i.e. a drug mechanistically directed to prevent myocyte death). Some agents have been reported to improve cardiac ailments significantly, but unfortunately they have been limited to experimental models of ischemia and reperfusion.

Antioxidants have been shown to cause apoptosis of both normal and transformed cells and deletion of the some of the redox active transcription factors leads to decreased survival suggesting that alterations in redox potential have major cellular consequences and that cellular function requires an optimal redox environment (Majno and Joris, 1995; Kumar et al., 2002). The central role of oxygen free radicals in the development of ischemic injury led to a widespread interest in the use of antioxidant therapy to attenuate ischemic injury. Antioxidants have been tested in several experimental and clinical models with mixed success. Measurement of ROS in vivo represents a complex challenge.

Recent attention has focused on the identification of indirect in vivo biomarkers of oxidative stress such as chemically stable, free radical catalyzed products of lipid peroxidation, modified proteins and indices of free radical catalyzed modification of DNA (Griendling and Fitzgerald, 2003). The major components of antioxidant system consist of antioxidant enzymes including superoxide dismutase (SOD), reduced glutathione (GSH), catalase and peroxidises. The role of antioxidant defence mechanism is illustrated (Figure 11-14).
SOD is a ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress by catalytic removal of superoxide radicals and conservation to \( \text{H}_2\text{O}_2 \) by the dismutation reaction. In cardiac myocytes, the mitochondrial enzyme manganese superoxide dismutase predominates, accounting for approximately 70% of the SOD activity in the heart and 90% of that in the cardiac myocyte (Aseem et al., 1997). MnSOD thus plays a major role in controlling mitochondrial ROS generated during normal oxidative phosphorylation. The role of SOD demonstrating reduction of oxidative stress and improvement in functional recovery of the heart. These antioxidative defences are overwhelmed during ischemia and after ischemic injury.

GSH is a tripeptide (γ-glutamylcysteinylglycine) that contains a free thiol group. GSH is a major tissue antioxidant that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water. Glutathione reductase generates GSH by the NADPH-dependent recycling of GSSG. GSH is an important soluble antioxidant that acts both to replenish GSHPx and as a direct scavenger of ROS and reactive nitrogen species. GSH is replenished the action of GR on GSSG and by de novo synthesis of GSH. The GSH/GSSG ratio, which is often used as an indicator of the cellular redox state, is >10 under normal physiological conditions (Griffith, 1999). GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells, but its value can be affected by other redox couples, including NADPH/NADP\(^+\) and thioredoxin \( \text{red}/\text{thioredoxin}_{\text{ox}} \) (Jones, 2002). Certain vitamins contribute to the cell antioxidant defence system. However, vitamins do not reduce the levels of ROS, but rather, act to protect target molecules.

Vitamin E (α-tocopherol) is the major lipid soluble antioxidant and requires prolonged and very high levels of oral treatment to achieve cardiac concentrations that are protective from ischemic injury. The importance of limiting myocardial ischemic
injury in clinical practice has been appreciated for more than three decades. Unfortunately, to date no therapeutic approach has been demonstrated clinically effective in salvaging heart muscle at risk of necrosis or improving contractile dysfunction. Several clinical studies have been shown that oxygen radical scavenging is effective in preventing reperfusion induced arrhythmias.

Antioxidant therapy reduces myocardial ischemic injury and the biochemical mechanisms involved in modulating apoptosis with a special reference to oxidative stress elicited signalling pathways. Previous studies with antioxidants and free radical scavengers have shown an inhibition of myocardial apoptosis. The exogenous administration of antioxidant substances or over expression of endogenous antioxidant enzymes ameliorates oxidative stress induced apoptosis in ischemic myocardium. The hypothesis that ROS are involved in induction of apoptosis has been supported by several experimental studies. It is now becoming clear that ROS generated during reperfusion are probably involved in electing myocardial apoptosis. Targeting oxidative stress is a promising and exciting new avenue in the treatment of IHD. Whether this will translate in clinical benefit for the patients with IHD remains to be determined. The interest in targeting oxidative stress in IHD has been shifted from the use of antioxidant vitamins to drugs specificity designed to inhibit the relevant radical producing enzymes and to cardiovascular drugs with free radical scavenging properties.

In fact, several cardiovascular drugs commonly used have demonstrated intrinsic antioxidant properties (Kopff et al., 2004). These include propranolol (Khaper et al., 1997); captoril (Hayek et al., 1998); losartan (Khaper et al., 2001); calcium channel blokers (Sevanian et al., 2000); statins (Delbosc et al., 2002); trimetazidine (Tselepis et al., 2001); aspirin (Lopez-Farre et al., 1996) and others. In addition, it has been suggested that the anti-inflammatory action of some of these drugs correlates with their antioxidant capacity.
Figure 11- Mitochondrial superoxide production and disposition
(Source: Hazel HS. The AAPS Journal, 2006)

Figure 12- Effect of oxidative stress and antioxidants in pathophysiology of ischemic injury in the heart
(Source: modified from Valko et al., 2007 Int J Biochem & Cell Biol).
Increase in oxidants or antioxidants results in a disturbance in cellular redox balance.

Oxidative stress occurs when oxidants exceed over the available antioxidants. In contrast, reductive stress occurs when antioxidants exceed over the oxidants present in the cell.

Figure 14- Illustration of the relationship between oxidants and antioxidants in the determination of cellular redox balance
(Source: Powers et al., J Sport sci, 2004).
2.9. Natural antioxidant and herbs: Potential agent for cardiovascular disorders

Commonly many foods and medicinal plants contain nonnutritive components such as flavonoids and other phenolic compounds that may provide protection against several chronic diseases through multiple effects, which are as yet poorly understood (Tanaka et al., 1993). These compounds may act as antioxidants by reacting with free radicals and thus interrupting the propagation of new free radical species, or by chelating metal ions such as Fe2+, which catalyze lipid oxidation to alter their redox potentials. In addition, it has been shown that antioxidant supplements can significantly improve certain immune responses (Hertog et al., 1993).

The function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at a level below which they will trigger the inflammatory cascade, a series of intracellular and intranuclear signaling that results in the release of destructive inflammatory cytokines (Rhee, 2006; Valko et al., 2007). In recent years, the traditional therapeutic approach to several chronic diseases has increasingly opened up to the contribution of antioxidant supplements, especially those from natural sources which have a higher bioavailability and therefore higher protective efficacy than synthetic antioxidants (Berger, 2005; Fusco et al., 2007; Herrera et al., 2009). The traditional Indian medicinal plants act as antiradicals and DNA cleavage protectors (Russo et al., 2001). The past two decades have witnessed several pharmacological interventions to limit ischemic injury.

The main aim of the research directed towards the science and pharmacology of ISP-induced ischemic injury is the discovery of drug that can be used in man to prevent ischemic injury and its sequel, for the betterment of the human health. Considerable experimental scientific and clinical effort, have been directed to achieve this goal.
Herbs are found to be a potent source of natural antioxidants. Some have been used for thousands of years and their clinical and pharmacological effects have been extensively studied from various viewpoints (Scartezzini and Speroni, 2000; Govindarajan et al., 2003). Herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life (Fugh-Berman, 2000). It is apparent that experimental evaluation of herbal drugs for the treatment of cardiovascular diseases is rather impressive, but very few have reached clinical trials and still fewer have been marketed. Hence, pharmacologists need to take more active interest in the evaluation of herbal drugs for potential activities and their standardization to allow them to be clinically effective and globally competitive.

2.10. Cardioprotective potential of medicinal plants

Recent years have witnessed a renewed interest in plants as pharmaceuticals worldwide. In the global context, herbal medicines flourish as the method of therapy of choice in many parts of the world and the increasing demand for herbal medicines is being fueled by a growing consumer interest in natural products. Now it is gaining new popularity as an alternative conventional medicine even in the industrialized countries and the adoption of crude extracts of plants for self medication by the general public is increasing.

Plant derived medicines are used in all civilizations and cultures and hence, plants have always played a key role in health care systems worldwide. In most developing countries, mainly in India and China, the indigenous modes of herbal
treatment are a part of the culture and the dominant method of healing therapy. These remedies, with a considerable extent of effectiveness, are socially accepted, economically viable and mostly are the only available source. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds. The World Health organization (WHO) estimates that 80% of the people of developing countries rely on traditional medicines, mostly plant derived drugs, for their health needs.

Indeed, ‘Phytomedicines’ are beginning to link traditional and modern medicines (Jain and Devasagayam, 2006). The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed (Walker, 1996). Many dietary antioxidants and some non nutrient based antioxidants from plants such as garlic, soy, green tea, red berries, tomatoes, grape seeds and several medicinal herbs mainly- Allium sativum; Aegle marmelos; Bacopa monnieri; Curcuma longa; Creategus oxycaynthia; Cucumis trigonus; Crocus sativus; Calotropis procera; Desmodium gangeticum; Erythrina stricta; Hibiscus rosa-sinensis; Hydrocotyle asiatica; Herba leonuri; Hemidesmus indicus; Inula racemosa; Ilex paraguariensis; Moringa olfera; Muntingia calabura; Ocimum sanctum; Picrrohiza kurroa; Salvia miltiorrhiza; Terminalia arjuna; Tinospora cardifolia; Vitis vinifera; Withania somnifera; Zingiber officinale etc have been investigated in recent years in animal models of myocardial infarction.

The prophylactic and therapeutic effects of many plants foods and extracts in reducing IHD have been demonstrated in several epidemiological, clinical and experimental studies (Hertog et al., 1993; Gupta et al., 2004; Arya et al., 2006;
Mohanty et al., 2008; Velavan et al., 2008; Nivethetha et al., 2009; Thippeswamy et al., 2009; Ojha et al., 2010; Hong et al., 2010; Sachdeva et al., 2010; Prince et al., 2010; Punithavathi et al., 2010; Kannan and Quine, 2011; Li et al., 2011; Parveen et al., 2011; Khandelwal et al., 2011; Shukla et al., 2012). These plants contain alkaloids, glycosides, terpenoids, lignins, phytoesterogens and flavonoids. Flavonoids are known to have numerous pharmacological effects which have been attributed to cardioprotective, radical scavenging, metal chelation and inhibition of calcium influx, regulation of cell signalling and modification of gene expression (Kong et al., 2001; Shukla et al., 2010).

These phytoconstituents have been reported to be active for the treatment of disorders of the peripheral circulation. Flavonoid based herbal medicines are used as cardioprotective agent as evidenced by large number of experimental and few clinical and epidemiological studies. Various studies have proved that the herbal extracts possesses cardioprotective effect against isoproterenol induced myocardial infarction and coronary artery ligated ischemia reperfusion injury in rats (Sharma et al., 2001; Gupta et al., 2004; Mohanty et al., 2004; Arya et al., 2006; Bansal et al., 2006; Mohanty et al., 2006). Literature survey reveals that previous investigators have demonstrated that medicinal herbs play a key role in protecting the heart from oxidative stress associated myocardial infarction and ischemia reperfusion injury by free radical scavenging, antioxidant, adaptogenic and anti inflammatory activity. However, several trails have failed to show the protective effect of antioxidants challenging the underlying principle of this hypothesis. Experimental and clinical studies have demonstrated that therapeutic interventions having antioxidants or free radical scavenging activity may exert cardioprotective effects against oxidative stress associated various cardiovascular diseases, including ischemic heart disease.
Table 2 - Medicinal plants and phytoconstituents evaluated so far for their cardioprotective activity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Medicinal Plants</th>
<th>Model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protykin (transresveratrol &amp; emodin)</td>
<td>In vitro</td>
<td>Sato et al., 2000</td>
</tr>
<tr>
<td>2</td>
<td>Ocimum sanctum</td>
<td>ISP</td>
<td>Sharma et al., 2001</td>
</tr>
<tr>
<td>3</td>
<td>Arjunolic acid</td>
<td>ISP</td>
<td>Sumitra et al., 2001</td>
</tr>
<tr>
<td>4</td>
<td>Allium sativum</td>
<td>ISP</td>
<td>Banerjee et al., 2003</td>
</tr>
<tr>
<td>5</td>
<td>Quercetin and gallic acid</td>
<td>In vitro</td>
<td>Yamashiro et al., 2003</td>
</tr>
<tr>
<td>6</td>
<td>Calotropis procera</td>
<td>ISP</td>
<td>Ahmed et al., 2004</td>
</tr>
<tr>
<td>7</td>
<td>Hydrocotyle asiatica</td>
<td>IR</td>
<td>Pragada et al., 2004</td>
</tr>
<tr>
<td>8</td>
<td>Salvia miltiorrhiza</td>
<td>In vivo</td>
<td>Gupta et al., 2004</td>
</tr>
<tr>
<td>9</td>
<td>Withania somnifera</td>
<td>ISP</td>
<td>Mohanty et al., 2004</td>
</tr>
<tr>
<td>10</td>
<td>Tinospora cardifolia</td>
<td>IR</td>
<td>Rao et al., 2005</td>
</tr>
<tr>
<td>11</td>
<td>Ilex paraguariensis</td>
<td>IR</td>
<td>Schinella et al., 2005</td>
</tr>
<tr>
<td>12</td>
<td>Herba leonuri</td>
<td>In vivo</td>
<td>Sun et al., 2005</td>
</tr>
<tr>
<td>13</td>
<td>Desmodium gangeticum</td>
<td>ISP</td>
<td>Kurian et al., 2005</td>
</tr>
<tr>
<td>14</td>
<td>Aegle marmelos</td>
<td>ISP</td>
<td>Prince et al., 2005</td>
</tr>
<tr>
<td>15</td>
<td>Ocimum sanctum</td>
<td>ISP</td>
<td>Sood et al., 2005</td>
</tr>
<tr>
<td>16</td>
<td>Ocimum sanctum</td>
<td>IR</td>
<td>Arya et al., 2006</td>
</tr>
<tr>
<td>17</td>
<td>Curcuma longa &amp; Ocimum sanctum</td>
<td>IR</td>
<td>Mohanty et al., 2006</td>
</tr>
<tr>
<td>18</td>
<td>Hibiscus rosa sinensis</td>
<td>ISP</td>
<td>Gauthaman et al., 2006</td>
</tr>
<tr>
<td>19</td>
<td>Lycopene</td>
<td>IR</td>
<td>Bansal et al., 2006</td>
</tr>
<tr>
<td>20</td>
<td>Zingiber officinale</td>
<td>ISP</td>
<td>Ansari et al., 2006</td>
</tr>
<tr>
<td>21</td>
<td>Curcuma longa</td>
<td>ISP</td>
<td>Mohanty et al., 2007</td>
</tr>
<tr>
<td>22</td>
<td>Rutin</td>
<td>ISP</td>
<td>Prince &amp; Karthick, 2007</td>
</tr>
<tr>
<td>23</td>
<td>Curcumin</td>
<td>ISP</td>
<td>Ansari et al., 2007</td>
</tr>
<tr>
<td>No.</td>
<td>Plant Name</td>
<td>Source</td>
<td>Reference</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------</td>
<td>--------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>24</td>
<td>Oleanolic Acid</td>
<td>ISP</td>
<td>Senthil et al., 2007</td>
</tr>
<tr>
<td>25</td>
<td>Silymarin</td>
<td>IR</td>
<td>Rao &amp; Viswanath, 2007</td>
</tr>
<tr>
<td>26</td>
<td><em>Bacopa monnieri</em></td>
<td>ISP</td>
<td>Nandav et al., 2007</td>
</tr>
<tr>
<td>27</td>
<td><em>Moringa olfera</em></td>
<td>ISP</td>
<td>Nandav et al., 2007</td>
</tr>
<tr>
<td>28</td>
<td><em>Curcuma longa</em></td>
<td>ISP</td>
<td>Mohanty et al., 2008</td>
</tr>
<tr>
<td>29</td>
<td><em>vitis vinifera</em></td>
<td>ISP</td>
<td>Velavan et al., 2008</td>
</tr>
<tr>
<td>30</td>
<td><em>Cucumis trigonus</em></td>
<td>ISP</td>
<td>Thippleswamy et al., 2009</td>
</tr>
<tr>
<td>31</td>
<td><em>Muntingia calabura L.</em></td>
<td>ISP</td>
<td>Nivethetha et al., 2009</td>
</tr>
<tr>
<td>32</td>
<td><em>Crocus sativus L.</em> (saffron)</td>
<td>ISP</td>
<td>Sachdeva et al., 2010</td>
</tr>
<tr>
<td>33</td>
<td><em>Erythrina stricta</em></td>
<td>ISP</td>
<td>Kuppusamy et al., 2010</td>
</tr>
<tr>
<td>34</td>
<td>Rutin</td>
<td>ISP</td>
<td>Prince &amp; Priya, 2010</td>
</tr>
<tr>
<td>35</td>
<td>Quercetin</td>
<td>ISP</td>
<td>Punithavathi &amp; Prince, 2010</td>
</tr>
<tr>
<td>36</td>
<td>Caulophine</td>
<td>LCA</td>
<td>Si et al., 2010</td>
</tr>
<tr>
<td>37</td>
<td>Curcumin</td>
<td>IR</td>
<td>Hong et al., 2010</td>
</tr>
<tr>
<td>38</td>
<td><em>Inula racemos</em></td>
<td>IR</td>
<td>Ojha et al., 2010</td>
</tr>
<tr>
<td>39</td>
<td>Ellagic acid</td>
<td>ISP</td>
<td>Kannan &amp; Quine, 2011</td>
</tr>
<tr>
<td>40</td>
<td>Phloroglucinol</td>
<td>IR</td>
<td>Li et al., 2011</td>
</tr>
<tr>
<td>41</td>
<td><em>Terminalia arjuna</em></td>
<td>ISP</td>
<td>Parveen et al., 2011</td>
</tr>
<tr>
<td>42</td>
<td><em>Hemidesmus indicus &amp; Hibiscus rosasinensis</em></td>
<td>IR</td>
<td>Khandelwal et al., 2011</td>
</tr>
</tbody>
</table>

In the present study, the medicinal plants were selected considering their traditional use, chemical constituents, biological and pharmacological properties such as antioxidants, cardioprotective, anti-inflammatory and anti-apoptotic activity. The hydroalcoholic extract of bark of *T. arjuna* and fruit pulp of *E. jambolana* was prepared, lyophilized and administered to rats for screening of their cardioprotective potential.
2.11. *Terminalia arjuna* (*T. arjuna*)

2.11.1 Distribution, Habitat and Ethnobotanical Consideration

*T. arjuna* (TA) is a deciduous and evergreen tree, standing 20-30m above ground level. It belongs to family Combretaceae (Chopra and Ghosh, 1929; Caius *et al*., 1930; Nadkarni and Nadkarni, 1954; Dwivedi S, 2007; Kumar *et al*., 2009). It is found in abundance throughout Indo-sub Himalayan tracts of Uttar Pradesh, Bihar, Madhya Pradesh, Delhi and Deccan region. It is also found in forests of Sri Lanka, Burma and Mauritius (Chopra *et al*., 1958). TA has huge trunk and horizontally spreading branches (Figure 17). The histology of *T. arjuna* bark reveals the presence of single layered epidermis with hair like projections and few scattered lenticels. Leaves are simple, borne sub opposite coriaceous, often crenulating, oblong or elliptic.

Among different species of *Terminalia*, the bark of *T. arjuna* has its own characteristic features and it has been reported to be beneficial in many cardiac ailments in ancient Indian medical literature, Ayurveda (Dwivedi S, 2007; Kumar *et al*., 2009; Maulik *et al*., 2012; Shukla *et al*., 2012). The bark leaves and fruits of *T. arjuna* have been used in indigenous system of medicine for different ailments (Warrier *et al*., 1996). The bark is said to be sweet, acrid, cooling and heating, aphrodisiac, expectorant, tonic, styptic, antidysenteric, purgative and laxative. Its use has been advocated in urinary discharge, strangury, leucoderma, anemia, hyperhidrosis, asthma and tumors. The bark powder has been attributed to possess cardioprotective properties (Dwivedi S, 2007; Kumar *et al*., 2009; Shukla *et al*., 2012; Maulik *et al*., 2012). Traditional method of its administration was to prepare an alcoholic decoction of its bark stem (asava) or give it along with clarified butter (ghrita) or along with boiled milk (kshirpak) (Nadkarni and Nadkarni, 1954; Warrier *et al*., 1996). Having realized the potential atherogenic properties of clarified butter
and whole milk it would be interesting to examine the role of such preparations in experimental model of atherosclerosis.

**Phytochemistry**

The phytochemistry of *T. arjuna* reveals the presence of arjunin, arjunetin, arjunic acid, arjunolic acid, arjungenin, arjunglicoside I and arjunglicoside II (Honda *et al.*, 1976). Later on a triterpene carboxylic acid, terminic acid, and arjunoside III and arjunoside IV were isolated from the ethyl acetate extract of its root (Anjaneyulu and Prasad, 1982).

![Chemical structure of terpenoids and glycosides found in *T. arjuna*: (a) arjunic acid, arjunoside I, arjunoside II and arjunoside IV; (b) arjunoside III; (c) terminic acid](source)

Figure 15

![Chemical structure of important flavonoids detected in *T. arjuna*: (a) arjunolone and bicaline; (b) apigenin and luteolin; (c) kempferol and quercetin; (d) pelargonidin and cyanidin](source)

Figure 16

(Source: Dwivedi S. J Ethnopharmacol, 2007).

(Source: Dwivedi S. J Ethnopharmacol, 2007).
Terminalia arjuna

Figure 17- Stem bark of *T. arjuna* and whole plant
(Source: Dwivedi S. J Ethnopharmacol, 2007)
2.11.2 Medicinal properties and uses

Several medicinal plants have been described to be beneficial for cardiac ailments in ‘Atharva Veda’ an ancient treatise from which Ayurveda, the Indian system of Medicine owes its origin (Shatvalekar, 1943; Dwivedi and Chaturvedi, 2000). *T. arjuna* is an important medicinal plant widely used in the preparation of ayurvedic formulations for over three centuries primarily as a cardiac tonic in India (Karthikeyan *et al.*, 2003). *T. arjuna* has been identified and researched for their putative lipid lowering and cardioprotective activities (Dwivedi, 1996). The bark stem powder of this tree has been mentioned to be useful for ‘hritshool’ (angina) and other related cardiac ailments by the ancient physicians (Gauthaman *et al.*, 2001; Parveen *et al.*, 2011; Oberoi *et al.*, 2011; Shukla *et al.*, 2012; Maulik *et al.*, 2012).

Recently there has been renewed interest in this plant because of its multimode cardioprotective activity. As there is no single drug till date which alone or in combination offers definite and reliable protection and/or cure from the ravages of atherosclerotic cardiovascular disorders. The time is ripe for evaluating the role of *T. arjuna* in the overall management of coronary artery disease (CAD) and related cardiovascular disorders.

**Cardiotonic activities**

*T. arjuna* has cardiotonic and stimulant actions (Dwivedi S, 2007). It was subsequently found that intravenous administration of the glycoside, obtained from the bark of *T. arjuna*, resulted in rise in blood pressure (Dwivedi S, 2007). The bark powder of TA, in addition to cardiotonic property, also possessed diuretic properties.
Review of Literature

(Caius et al., 1930). Subsequent experimental studies in isolated frog heart revealed that the aqueous extract of the bark had chronotropic and inotropic activities (Chopra et al., 1958).

**Cardioprotective and antioxidant activities**

The cardioprotective effect of *T. arjuna* has been studied in ISP-induced myocardial ischemia model in rats, rabbits and mice by several authors (Sumitra et al., 2001; Gauthaman et al., 2001; Karthikeyan et al., 2003; Dwivedi et al., 2007; Kumar et al., 2009; Maulik et al., 2012; Shukla et al., 2012). The onset of myocardial ischemia and its severity both were reduced in rabbits pre-treated with *T. arjuna* compared to placebo control rabbits (Dwivedi et al., 1988). Cardioprotection conferred by arjunolic acid could possibly be due to the protective effect against the damage caused by myocardial necrosis (Sumitra et al., 2001; Kumar et al., 2009; Shukla et al., 2012). Role of *T. arjuna* as an antioxidant agent on ischemic perfused rat heart has been studied very recently (Kumar et al., 2009; Parveen et al., 2011; Shukla et al., 2012). Based on the results of the various studies, it was thus concluded that crude bark of *T. arjuna* augments endogenous antioxidant compounds of rat heart and prevents it from oxidative stress (Gauthaman et al., 2001; Karthikeyan et al., 2003). Arjungenin an oleanane terpenoid derived from *T. arjuna* bark and its glucoside, arjunglucoside II, have been demonstrated to exert free radical scavenging activities in human polymorphonuclear cells (Pawar and Bhutani, 2005). Aqueous extract of *T. arjuna* bark has been demonstrated to protect the oxidant damage to the liver and kidney following carbon tetrachloride challenge in mice (Manna et al.,
2006) and N-nitrosodiethylamine-induced liver injury in rats (Sivalokanathan et al., 2006). These observations clearly indicate endogenous antioxidant activity of the *T. arjuna*.

**Effect on endothelial dysfunction**

On account of its rich bioflavonoid content it is considered to be a strong antioxidant and an ideal agent for correction of endothelial dysfunction. Working on this hypothesis its effect on endothelial-dependent flow mediated and endothelium independent nitroglycerine (NTG) mediated dilatation was studied in smokers and non-smoker healthy males (Bharani et al., 2004).

**Toxicity and side effects**

*T. arjuna* has been used in the dose of 1-2 g / day in various clinical studies. This has been found to be the optimum dose in patients of CAD. At this dosage, it is well tolerated and has fewer side effects like mild gastritis, headache and constipation. No haematological, metabolic, renal and hepatic toxicity has been reported even more than 24 months of its administration (Dwivedi et al., 1989; Dwivedi and Agarwal, 1994; Bharani et al., 1995). In a study the aqueous extract of its bark could protect the liver and kidney tissues of mice against carbon tetra chloride (CCl4) - induced oxidative stress probably by increasing antioxidant defence activities (Manna et al., 2006). In another study using ethanolic extract, 500 mg/kg dose of its bark stem in alloxan-induced diabetic rats, it was found that the drug reduced the lipid peroxidation and raised endogenous antioxidant enzymes in liver and kidney tissues (Raghvan and Kumari, 2006).
2.12. **Eugenia jambolana (E. jambolana)**

2.12.1 **Distribution, Habitat and Ethno botanical consideration**

The evergreen plant of *E. jambolana* is originally from Indonesia and India. Indian mythology describes the Indian subcontinent as an island, 'situated in the centre of the world and called Jambudweep. Due to a majority of Jamun (black berry) trees, this island was named as Jambudweep. *E. jambolana* is evergreen tropical tree, 50 to 100 ft tall with oblong opposite leaves that are smooth and glossy, has a terpentine smell. *E. jambolana* has fragrant white flowers in branched clusters at stem tips and purplish-black oval edible berries. *E. jambolana* (Figure 18) belonging to the family Myrtaceae is a large evergreen tree indigenous to the Indian subcontinent. However today these trees are found growing throughout the Asian subcontinent, Eastern Africa, South America, Madagascar and have also naturalized to the warmer regions of the United States of America mainly in Florida and Hawaiï (Warrier *et al.*, 1996; Li *et al.*, 2009). The trees are famous for their fruits and their colloquial names, which include Java plum, Portuguese plum, Malabar plum, black plum, Indian blackberry, jaman, jambu, jambul are attributed to the fruits (Warrier *et al.*, 1996).

There are two main morphotypes of *E. jambolana* in Indian subcontinent and this is based on the morphological and organoleptic features, the Kaatha jamun which are small and acidic to taste, and the ras Jaman, that are oblong, dark-purple or bluish, with pink, sweet fleshy pulp and small seeds (Morton, 1987; Jabbar *et al.*, 1994). The trees grow up to a height of 50 ft and have large canopy. The young bark is pale brown in colour, while the mature are darkish brown and scaly. The leaves are elliptic to broadly oblong, smooth, glossy, leathery and fibrous in nature. The trees flowers once in a year and in the Indian subcontinent it is mostly during the month of June-July. The fruits are found in clusters of four to twenty and do not ripen.
simultaneously. Each fruit is round or oblong or ellipsoid, long with a centrally placed large seed.

The fruit pulp of *E. jambolana* is a rich source of anthocyanins and flavonoids (Quercetin, Myricetin and Kaempherol), raffinose, glucose, fructose (Srivastava, 1953), citric acid, mallic acid (Lewis *et al.*, 1956), gallic acid, anthocyanins (Jain and Seshadri, 1975).

![Figure 18- E. jambolana fruits with their leaves](Source: Baliga *et al.*, Food Res Int 2011)
Phytochemistry of *E. jambolana*

*E. jambolana* is known to possess diverse phytochemicals such as β-sitosterol, betulinic acid, mycamimose, crategolic (maslinic) acid, n-heptacosane, n-nonacosane, n-hentriacontane, nootcasanol, n-triactanol, n-dotricontanol, quercetin, myricetin, myricitirin and the flavonol glycosides myricetin 3-O- (4″-acetyl) -α-L-rhamnopyranosides, acylated flavonol glycosides (Mahmoud *et al.*, 2001; Sagrawat *et al.*, 2006). The essential oil from the leaves is shown to contain the phytochemicals pinocarveol, α-terpeneol, myrtenol, eucarvone, muurolol, α-myrtenal, cineole, geranyl acetone, α-cadinol and pinocarvone (Shafi *et al.*, 2002). The stem bark and fruit pulp is reported to possess friedelin, friedelan-3-α-ol, betulinic acid, β-sitosterol, kaempferol, β-sitosterol-D-glucoside, gallic acid, ellagic acid etc. Active molecules found in *E. jambolana* have been illustrated below (Figure 19).

![Figure 19](image-url)

**Figure 19** - Presence of various phytoconstituents a. aliphatic acids; b. anthocyanins; c. flavonoids; d. important phenolics; e. phytosterols and terpenes in *E. jambolana*.
(Source: Baliga *et al.*, Food Res Int 2011).
2.12.2 Medicinal properties and uses

In traditional medicine *E. jambolana* has been indicated in Ayurveda, an ancient system of Indian medicine, for treatment of diabetes mellitus (Chopra *et al.*, 1958). All parts of the tree and the seeds in particular, have a long history of medicinal use in the various folk systems of medicine in countries where *E. jambolana* is reported to grow (Bhandary *et al.*, 1999). *E. jambolana* is also used extensively in the various traditional systems of medicine viz. Ayurveda, Unani, Siddha, Srilankan, Tibetan and in the Homeopathy systems of alternative and complementary medicine (Warrier *et al.*, 1996). Before the discovery of insulin, *E. jambolana* was useful in the treatment of diabetes and was used either alone or in combination with other hypoglycemic plants even in Europe (Helmstädter, 2007). According to Ayurveda, their barks are acrid, digestive and astringent. They are supposed to be useful for treating sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers (Warrier *et al.*, 1996). The ash of the leaves is effective in strengthening the teeth and the gums. The bark is also known to possess wound healing properties. In the Siddha system of medicine, *E. jambolana* is considered to be a haematinic, semen promoting and to decrease excessive heat of the body (Warrier *et al.*, 1996). According to the Unani system of medicine, they are supposed to be a liver tonic, to enrich blood, strengthen teeth and gums. The decoction is supposed to be a good lotion for removing ringworm infection of the head (Warrier *et al.*, 1996).

The fruit pulp of *E. jambolana* is a rich source of anthocyanins and flavonoids. The flavonoids including Quercetin, Myrcetin and Kaempferol have been shown to
exhibit a series of biological effects. They include: inhibition of lipid peroxidation and platelet aggregation which contributes to reduced thrombotic tendencies (Venkateswarlu, 2006) and also cholesterol lowering effects by alteration in cholesterol absorption, triglycerides assembly and processing of lipoproteins in plasma. Multiple functions of dietary polyphenols help in reduction of coronary heart disease risk by improving plasma lipid profile (Zern and Fernandez, 2005). Studies have shown that the pulp of *E. jambolana* is highly nutritive and contains important minerals like sodium, potassium, calcium, phosphorous, iron and zinc; water soluble vitamins like ascorbic acid, thiamine and niacin; carbohydrates like glucose, mannose, sucrose, maltose, fructose, galactose and mannose; free amino acids like alanine, asparagine, tyrosine, glutamine and cysteine (Noomrio and Dahot, 1996; Paul and Shaha, 2004). The sourness of fruits of *E. jambolana* may be due to presence of gallic acid.

Gallic acid, an endogenous plant phenol is found abundantly in tea, grapes, different berries, fruits as well as wine (Ma *et al*., 2003; Singh *et al*., 2004). Gallic acid (3,4,5-trihydroxybenzoic acid), a metabolite of propyl gallate is known to potentiate several pharmacological and biochemical pathways having strong antioxidant (Kim *et al*., 2002), anti-inflammatory (Kroes *et al*., 1992), antimutagenic (Gichner *et al*., 1987) and anticancer activity (Mirvish *et al*., 1975; Inoue *et al*., 1995).

**Free radical scavenging and antioxidant effects**

Reports indicate that plants rich in anthocyanins, flavonoids and polyphenols are observed to be effective in scavenging the free radicals (Sánchez *et al*., 1999; Alia *et al*., 2008; Rufino *et al*., in press). With regard to *E. jambolana*, Benherlal and
Arumughan (2007) evaluated the antioxidant effects of the ethanolic extract of fruit pulp, kernel and seed coat in various in vitro assays with gallic acid, quercetin and trolox as reference molecules. In the hydroxyl radical scavenging assay, the kernel extract was comparable to the effect of catechin (Benherlal and Arumughan, 2007).

**Inhibition of lipid peroxidation**

Sultana et al (2007) evaluated the antioxidant activity of the 80% methanol, ethanol and acetone (solvent: water, 80:20 v/v) extract of the bark by measuring the reducing power, inhibition of peroxidation using linoleic acid system and DPPH scavenging activity. Benherlal and Arumughan (2007) evaluated the inhibition of lipid peroxidation of the ethanolic extract of the fruit pulp, kernel and seed coat in vitro, and observed that the kernel extract was better than the seed coat and pulp extract, but less than the reference molecules (gallic acid, quercetinm and trolox). Veigas et al (2007) studied the ability of anthocyanin rich pulp extract of *E. jambolana* for its efficacy to inhibit the iron (FeSO4)-induced lipid peroxidation in the various organs (rat brain, liver, liver mitochondria, testes and human erythrocyte ghost cells) in vitro and observed it to be effective in all the organs but with differential degree.

**Cardioprotective effects**

Animal studies have also shown that administering *E. jambolana* decreased the levels of lipid peroxides (Chaturvedi et al., 2007 and 2009) in the brain, heart, liver, kidneys and serum of diabetic animals, suggesting its usefulness in amelioration of diseases (Prince et al., 1998; Ravi et al., 2004; Prince et al., 2004; Ravi et al., 2005; Chaturvedi et al., 2009; Tanwar et al., 2011).
Anti-inflammatory effects

Several reports have suggested that diseases associated with inflammation may be ameliorated by *E. jambolana*. Chloroform fraction of the seed inhibited the carragecin, kaolin and other inflammatory mediator induced edema in rats (Chaudhuri *et al*., 1990).

Gallic acid present in the fruit pulp of *E. jambolana* reported to show anti-inflammatory responses in vitro and in vivo models (Kim *et al*., 2006). Gallic acid suppresses pro-inflammatory cytokine production in murine peritoneal macrophages (Matsuo *et al*., 1997; Kwon *et al*., 2004). The inhibitory effect of gallic acid on the pro-inflammatory cytokine was nuclear factor kB and p38 mitogen activated protein kinase dependent.

Hepatoprotective effects

Studies have also shown that the hepatoprotective effects of the pulp of *E. jambolana* also extended against the paracetamol-induced hepatotoxicity in rats (Das and Sarma, 2009). Decrease in the serum levels of ALT, AST, ALP and total bilirubin that were elevated in the paracetamol alone cohorts (Das and Sarma, 2009). The histopathological findings showed a reduction in necrosis and fibrosis (Das and Sarma, 2009).

Antidiabetic activities

*E. jambolana* has been thoroughly investigated for its antidiabetic effects both in preclinical and human studies (Helmstädter, 2008). Many studies in the past two decades have shown that the seed (Achrekar *et al*., 1991; Rathi *et al*., 2002; Sharma *et al*., 2003; Ravi *et al*., 2004 and 2005; Sridhar *et al*., 2005; Sharma *et al*., 2008; Panda
The fruit pulp (Achrekar et al., 1991; Pepato et al., 2005; Sharma et al., 2006; Sundaram et al., 2009) and bark (Villasenor and Lamadrid, 2006) possess antihyperglycemic effects, while the leaf was ineffective and devoid of this pharmacological effects (Pepato et al., 2001). The seed has been subjected to detailed investigations and observations suggest that it is effective when given as powder (Sridhar et al., 2005) or as an extract (Achrekar et al., 1991; Sharma et al., 2003; Ravi et al., 2004 and 2005; Sharma et al., 2008; Panda et al., 2009; Sundaram et al., 2009) in reducing both hyperglycemia and diabetic complications in the experimental animals.

_E. jambolana_ is shown to be effective in streptozotocin (Ravi et al., 2004 and 2005; Sridhar et al., 2005; Sharma et al., 2008; Sundaram et al., 2009; Panda et al., 2009) and alloxan (Rathi et al., 2002; Kar et al., 2003; Sharma et al., 2003 and 2006) induced models of Type 1 diabetes as well as in the fructose-induced model (Vikrant et al., 2001; Suganthi et al., 2007) for type 2 diabetes.

In our lab, the active compound (FIIc) of _E. jambolana_ has already been isolated (US Patent no. 6,426,826, August, 2002; Indian Process Patent no. 1, 88,759 May, 2003; Indian Product Patent no. 2, 30,753 February, 2009), which has strong anti-diabetic property (Sharma et al., 2003 and 2006).