introduction & review of literature
INTRODUCTION AND REVIEW OF LITERATURE

Healthy human life is always cardinal for human being starting from his birth to the end of the life. The number of diseases plays a key role in disturbing the healthy human life. Along with modernisation as well as sophistication in the life, human health directly or indirectly faces challenge from several diseases sometime in survival and sometimes in surrender to diseases. Cardiovascular diseases (CVDs) are a heterogeneous group of disorders that affect the heart and blood vessels. Among these, the ischemic heart disease (IHDs) is the leading cause of morbidity and mortality in a worldwide epidemic.

Myocardial ischemia (MI) in particular, is one of the main causes of death from CVDs (Aman et al., 2011). MI has emerged as a major health problem accounting for roughly 20% of all worldwide deaths per year (Karthick and Prince, 2006) and is predicted that by the year 2020 this diseases will persist as the major and the most common threat to human life (Mahendra et al., 2010).

MI, commonly known as heart attack is a disease that occurs when the blood supply to a part of the heart is interrupted, causing the death of heart tissue. It may present as a typical heart attack, as sudden death, or it may be detected at an advanced stage and be described as silent infarct. It is characterised by an imbalance between myocardial oxygen supply and demand, causing cardiac dysfunction, arrhythmias, myocardial infarction and sudden death. Various clinical ischemic manifestations are caused by obstruction of coronary blood flow by coronary stenosis, thrombosis and/or hyperconstriction (vasospasm) of epicardial and microvascular coronary arteries which also results in the necrosis of the myocardium (Hiroaki and Satoshi, 2008).
Introduction and Review of Literature

According to World Health Organisation (WHO) data, 16.7 million people die each year owing to heart attacks. The figure is one third of the number of deaths worldwide. By 2020-30 more deaths will be caused by heart attacks and India will lead in the number of such deaths in the world (Gupta and Gupta, 1996). In developed countries, IHD is predicted to rise 30-60% between 1990 and 2020. In developing countries, 60% of the world’s patients with heart disease are predicted to live in India by 2010 (Murray and Lopez, 1997) and the rates are predicted to increase by 120% in women and 137% in men from 1990 to 2020 (Ghaffar et al., 2004). Gupta et al., (2008) reported the rising prevalence of IHD in India from 2000-2020.

Table 1: Ischemic heart disease deaths in India and China in millions.

<table>
<thead>
<tr>
<th>Disease</th>
<th>2000</th>
<th>2010</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>India</td>
<td>China</td>
<td>EME</td>
</tr>
<tr>
<td>Ischemic heart diseases (IHD)</td>
<td>1.59</td>
<td>0.99</td>
<td>1.84</td>
</tr>
<tr>
<td>Cardiovascular diseases (CVDs)</td>
<td>3.01</td>
<td>3.30</td>
<td>3.49</td>
</tr>
</tbody>
</table>

EME: Established market economies

Overall burden of CVD in India

Leeder et al., (2004) estimated total years of life lost due to cardiovascular disease (CVD) among the Indian men and women aged 35- 64 to be higher than comparable countries such as Brazil and China, as demonstrated in Table 2. These estimates are predicted to increase from year 2000 to 2030, when the differences may become more marked.

Table 2: Estimates of total years of life lost due to cardiovascular diseases (CVDs) in 2000 and 2030.

<table>
<thead>
<tr>
<th>Country</th>
<th>2000 Total years life lost</th>
<th>Rate per 100,000</th>
<th>2030 Total years life lost</th>
<th>Rate per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>9,221,165</td>
<td>3,572</td>
<td>17,937,070</td>
<td>3,707</td>
</tr>
<tr>
<td>Brazil</td>
<td>1,060,840</td>
<td>2,121</td>
<td>1,741,620</td>
<td>1,957</td>
</tr>
<tr>
<td>China</td>
<td>6,666,990</td>
<td>1,595</td>
<td>10,460,030</td>
<td>1,863</td>
</tr>
</tbody>
</table>
Types of myocardial ischemia

MI is a well defined pathological occurrence that can result in four main clinical manifestations: stable angina, unstable angina, prinzmetal's angina, myocardial infarction. MI is caused by an insufficient blood supply to the myocardium (due to either increased substrate demand by the myocardium and/or to the narrowing/closure of a/several coronary artery/arteries). In most cases the narrowing of a coronary artery is the decisive factor for this condition. It can be produced by spasm, atherosclerotic plaques, thrombotic occlusion, or a variable combination of these. Sudden cardiac death subsequent to myocardial ischemia has been mainly attributed to malignant arrhythmias such as ventricular tachycardias and ventricular fibrillation (Gasser et al., 1994).

Stable angina

Stable angina refers to cardiac ischemia that results when oxygen demand of the heart exceeds the blood supply available. One of the most common causes of stable angina is deposition of cholesterol plaques in the arteries (atherosclerosis) that narrow the blood vessels and decrease the amount of blood that can flow inside. The unique symptoms include chest discomfort or pain that usually brought on by exertion or emotion (Becker et al., 2008).

Unstable angina

In unstable angina, the demand for oxygen by the heart is unchanged; instead the supply to the heart is decreased. This can lead to a heart attack and symptoms include chest pain at rest, new onset chest pain that is worsening and not improving. Causes include rupture of preexisting cholesterol plaques that block the vessel downstream or hemorrhage (Montalescot et al., 2009).

Prinzmetal's angina

This is a unique form of cardiac ischemia that is characterised by transient constriction (also known as vasospasm) of the arteries that supply the heart. This results in temporary decrease in blood to the heart, with symptoms of chest pain that occur at rest and is also associated with disruptions of heart rhythm. This can be easily treated with medications that cause dilation of the coronary arteries, such as calcium-channel blocker type drugs (Keller and Lemberg, 2004).
Myocardial infarction

Myocardial infarction, also known as heart attack, occurs when heart tissue dies as a result of decreased blood supply. This often begins as unstable angina and progresses if not treated promptly to a clinical heart attack. The symptoms include chest pain, nausea, vomiting, sweating, radiating pain to the jaw and/or left arm and a sense of impending doom (Thygesen et al., 2007).

Signs and symptoms

The following conditions have been closely associated with myocardial ischemia.

- High blood pressure
- Poor circulation
- Chest pain
- Foot and leg pain
- Shoulder and arm pain
- Muscle pain
- Neck or jaw pain
- Speech difficulty
- Vertigo
- Clammy skin
- Nausea
- Double vision
- Numbness on one side

In silent ischemia, some people do not experience any signs and symptoms of myocardial ischemia. Symptoms of cardiac ischemia can be classified as typical and atypical and it is helpful to know the duration of those symptoms to aid the diagnosis. Symptoms that last briefly few minutes to less than an hour are more often associated with angina. If the symptoms do not readily resolve with rest and continue for hours, even if intermittently, it is suggestive of a myocardial infarction.

Typical symptoms

Chest pain, especially pain that develops during physical exertion, such as running or during sex, but that resolves with rest, suggests ischemia. Chest pain can vary in its location and intensity. Some patients describe the experience as a
Introduction and Review of Literature

tightening in the middle of the chest or severe pressure. It may be localized over the upper left chest, it may also radiate to the back or it can mimic heartburn (Mitchell et al., 2007). Accompanying symptoms include diaphoresis (sweaty palms, clammy skin), nausea and vomiting or shortness of breath (dyspnea). Pain radiating to the jaw or down the left arm, heart palpitations and dizziness are also symptoms of ischemia. Respiratory issues, gastrointestinal conditions, anxiety and musculoskeletal problems in the muscles overlying the chest or between the ribs can cause similar complaints.

Atypical symptoms

Atypical symptoms are seen in women, diabetics and the elderly more frequently than other demographics. Atypical symptoms may include abdominal pain, heartburn, anxiety or simply fatigue. Ischemia also can occur without any noticeable symptoms, a condition known as silent ischemia (Mitchell et al., 2007).

Risk factors for MI among Indians and South Asians

The contributing factors for the growing burden of IHDs are in prevalence of risk factors, especially hypertension, diabetes, overweight or obesity, physical inactivity, elevated low density lipoproteins, triglycerides, reduced high density lipoproteins, increased blood cholesterol, male gender, family history of heart diseases and use of tobacco (Smith et al., 2004).

Factors that may increase the risk of developing myocardial ischemia include:

Tobacco

Smoking and long term exposure to secondhand smoke damage the interior walls of arteries, allowing deposits of cholesterol and other substances to collect and slow blood flow. Smoking also increases the risk of blood clots forming in the arteries that can cause myocardial ischemia.

Diabetes

Diabetes is the inability of the body to adequately produce or respond to insulin properly. Insulin, a hormone secreted by the pancreas, allows the body to use glucose, which is a form of sugar from foods. Diabetes can occur in childhood, but it appears more often in middle age and among overweight people. Excess sugar in the bloodstream increases the risk of MI and other heart problems.
High blood pressure

Over time, high blood pressure can damage arteries that feed heart by accelerating atherosclerosis. The risk of high blood pressure increases with age, but the main culprits for most people are eating a diet too high in salt and being overweight. High blood pressure can also be an inherited problem.

High blood cholesterol or triglyceride levels

Cholesterol is a major part of the deposits that can narrow arteries throughout the body, including those that supply to heart. Low density lipoprotein (LDL) cholesterol (the "bad" cholesterol) is most likely to narrow arteries and increases the risk of myocardial ischemia. A high LDL level is undesirable and is often a result of a diet high in saturated fats and cholesterol. A high level of triglycerides, a type of blood fat related to diet, also is undesirable.

Lack of physical activity

An inactive lifestyle contributes to high blood cholesterol levels and obesity. People who get regular aerobic exercise have better cardiovascular fitness, which decreases their risk of MI. Exercise also lowers high blood pressure.

Obesity

Obese people have a high proportion of body fat, often with a higher body mass index (BMI) (≥30). Obesity raises the risk of MI because it's association with high blood cholesterol levels, high blood pressure and diabetes.

Family history

Family history of heart attack or coronary artery disease, may also contributes to increased risk of MI.

Odds ratios for common risk factors for myocardial ischemia

India; specific adjusted odds ratios (OR) and adjusted population adjusted risk (PAR) for common risk factors for acute myocardial infarction (AMI) are shown in Table 3. All eight (8) risk factors combined for an odds ratio of 123.3 (95% CI: 38.7-400.2) for MI in South Asians with a PAR of 85.8% (95% CI: 78.0-93.7%). Joshi et al., (2007) reported the odds ratios and population adjusted risk for AMI in Indian population enrolled in the INTERHEART study.
### Introduction and Review of Literature

#### Table 3: Common risk factors for myocardial ischemia in Indian population.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>India</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current and former smoking</td>
<td>34.6</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>3.39 (2.62 to 4.36)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>43.3 (36.2 to 50.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11.4</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.64 (1.90 to 3.65)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>13.8 (9.5 to 19.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11.9</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.03 (1.45 to 2.84)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>8.3 (4.6 to 14.5)</td>
</tr>
<tr>
<td>High waist-to-hip ratio</td>
<td>19.5</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>4.29 (3.09 to 5.93)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>52.0 (42.5 to 61.3)</td>
</tr>
<tr>
<td>Stress or depression</td>
<td>76.5</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.57 (0.95 to 6.65)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>NA</td>
</tr>
<tr>
<td>Exercise intensity</td>
<td>6.8</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.58 (0.34 to 1.01)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>36.5 (12.8 to 69.4)</td>
</tr>
<tr>
<td>Alcohol consumption ≥ once/week</td>
<td>15.3</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.64 (1.21 to 2.27)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>-47.1 (-82.6 to -11.5)</td>
</tr>
<tr>
<td>Apolipoprotein B/apolipoprotein A-I ratio</td>
<td>36.5</td>
</tr>
<tr>
<td>Controls, %</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.31 (1.33 to 3.99)</td>
</tr>
<tr>
<td>PAR (95% CI)</td>
<td>49.0 (29.3 to 69.0)</td>
</tr>
</tbody>
</table>

MI which arises out of a lot risk factors working in concert, gives rise to a lot of unfavorable metabolic outcomes. The end result of which is the ultimate morbidity of the patient or even death.

**Diagnosis of myocardial ischemia**

IHD is the number one cause of mortality and morbidity, the prevalence of disease is expected to increase further. This unfortunate reality is unlikely to change
in the near future (Lampe et al., 2005). IHD is a complex syndrome with a heterogeneous etiology and a physiologic continuum of events progressing from plaque instability to plaque rupture, intracoronary thrombus, reduced coronary blood flow, MI, reversible damage and necrosis (Fuster et al., 1992; Perers et al., 2004). It is well known that the clinical signs and symptoms of individuals (patients) presenting with suspected IHDs are frequently vague and non-specific and mimic a number of other conditions. Most of these events are clinically unrecognizable and biochemically undetectable until the onset of necrosis. In addition, the sensitivity of markers is time dependent and even the more highly sensitive and specific markers often give false negative results on admission.

The diagnostic approach to MI remains one of the most difficult and controversial medical challenges (Rosalki et al., 2004). For this reason, measurement of biomarkers should be obtained in all individuals (patients) presenting with IHD symptoms. Although quantitative measurement of biochemical markers is important for objectifying the diagnostic process of the myocardial ischemia workup. Electrocardiogram (ECG) must be used in conjunction with biomarkers in the diagnostic evaluation of suspected MI (Robert, 2007). The WHO developed a definition of AMI, which requires the presence of two of the following major criteria: chest pain and indicative laboratory changes (Rosalki et al., 2004). The concept of integrating clinical, electrocardiographic and laboratory data was a major innovation and by the early 1990s the WHO definition had become a cornerstone in clinical and laboratory medicine.

Despite increasing focus on biochemical markers during the last two decades, the search for the optimal marker is ongoing. Ideally a biochemical marker of MI should have the following properties: a considerable concentration in the myocardium; absence from non-myocardial tissue and normal serum; rapid release into the blood at the time of ischemia; a relationship to the extent of injury; and persistence in the blood for a sufficient length of time to provide a diagnostic window. In addition, the test should be rapid, easy to perform and inexpensive. Early markers of myocardial ischemia, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were non-specific and did not provide definitive proof of myocardial involvement.
Lactate dehydrogenase (LDH)

Lactate dehydrogenase transfers hydrogen using NAD⁺ as hydrogen acceptor thus catalysing the oxidation of L-lactate to pyruvate. LDH activity is present in all the cells of the body predominantly in cytoplasm of the cell. Thus tissue levels are greater than those in serum, thus even a small mass of damaged tissue causes leakage of enzyme and increasing its level in serum significantly (Burtis and Ashwood, 1999). Measurement of LDH isoenzymes is necessary for greater specificity for myocardial injury. There are 5 isoenzymes (1 through 5). Ordinarily, isoenzyme 2 is greater than 1, but with myocardial injury, this pattern is “flipped” and 1 is higher than 2.

The normal ratio of LDH-1 to LDH-2 is <0.7. In MI the ratio increases to >1 and is rarely greater than 1.3. LDH-1 to LDH-2 rise above base line at around 10 hours following MI, peak at about 24 to 48 hours and stay elevated in blood for up to 14 days post MI (Conti, 1999). LDH-5 from liver may be increased with necrosis from passive congestion with heart failure following ischemic myocardial injury (Eisenman, 2006; Braunwald et al., 2002; Aviles et al., 2002).

Creatine kinase (CK)-MB

Creatine kinase (CK) catalyses the transfer of high energy phosphate from adenosine triphosphate to produce creatine phosphate. Creatine kinase is not excreted in the urine and its levels are not influenced by changes in renal or hepatic blood flow in animals. Inactivation occurs in the lymphatics by proteolysis. The classification of isoenzymes is based on the presence of two subunits (M and B). Creatine kinase MM is located mainly in striated muscle, CK-BB is most abundant in the brain and CK-MB is found in the heart (Trask and Billadello, 1990).

The majority of patients undergoing cardiac operation show an abnormal CK-MB release (Griesmacher et al., 1990) within 6 to 8 hours, returning to normal within 2 to 3 days. This profile is seen earlier than after acute myocardial infarction. In addition to these isoenzymes, isoenzyme subforms, known as isoforms, have been identified (MB1, MB2). The absolute serum level of CK-MB2 and the ratio of CK-MB2 to CK-MB1 allow earlier and more precise diagnosis of perioperative myocardial injury compared with CK-MB (Hamm and Katus, 1995). Assays to measure the enzymatic activity of the CK-MB isoenzyme and the derived CK:CK-
MB ratio was important advances, especially in terms of improved specificity (Karras and Kane, 2001). The introduction of immunologic mass determination of CK-MB was a major breakthrough that replaced the traditional enzymatic assay. CK-MB is the standard against which new biochemical markers are compared with respect to diagnostic accuracy and quantification of myocardial necrosis. Although this innovation did not lead to substantial advances in clinical management, it addressed most of the technical and analytical shortfalls of the earlier CK-MB enzymatic activity tests.

Ischemia modified albumin (IMA)

The discovery that albumin, in the serum of patients with myocardial ischemia, exhibited lower metal binding capacity for cobalt than the albumin in serum of normal subjects was originally made by Bar-Or et al., (2001). Based on these observations, an assay was recently developed in which the cobalt not sequestered at the N-terminus of albumin is detected using a colorimetric indicator. Ischemia modified albumin (IMA), as measured by the albumin cobalt binding assay, has been recently proposed for early detection of myocardial ischemia without infarction (Christenson et al., 2001; Roy et al., 2004a; Roy et al., 2004b; Anwaruddin et al., 2005). During ischemia, albumin’s capacity to bind with transition metals like cobalt is reduced, probably due to modification of N-terminal metal binding domains on the albumin moiety, especially at the aspartyl–alanyl–histidyl–lysine sequence (Gidenne et al., 2004).

Lippi and colleagues recently speculated that in vivo generation of IMA could be interpreted as an efficient endogenous response to ischemia, preventing myocardial damage or limiting the extent of myocyte necrosis (Lippi et al., 2006). In clinical studies, IMA has been shown to rise within minutes after the onset of ischemia, remain elevated for 6 to 12 hours and return to baseline within 24 hours (DeFilippe et al., 2003). Based on this premise, any IMA increase might be interpreted as an indicator of ischemia before necrosis, displaying up to 90% negative predictive value at the conventional diagnostic threshold. When results of the IMA assay are added to a traditional panel CK-MB the diagnostic sensitivity for myocardial ischemia increases from 57% to as high as 97%. Studies have shown that IMA is highly sensitive for the identification of MI and in combination with the ECG has both high
sensitivity and negative predictive value (Spyridon et al., 2006). A non-diagnostic IMA value could perhaps be part of a screening and exclusion strategy for MI. IMA is a marker of impending myocyte necrosis. During ischemia, free radical damage alters the ability of albumin to bind cobalt. Using a colour indicator (dithiotreitol) to detect added cobalt, the level of such altered albumin in serum can be quantitated (Bar-Or et al., 2001). Sinha et al., (2004) evaluated IMA in conjunction with ECG changes in 208 patients presenting to the emergency department within three hours of the onset of acute chest pain. In the whole patient group, sensitivity of IMA at presentation for an ischemic origin of chest pain was 82%, compared with 45% of ECG. IMA used together with ECG, had a sensitivity of 92%, respectively.

Similarly, Roy et al., (2004a) showed that in 131 patients presenting to the emergency department with symptoms suggestive of acute myocardial ischemia but with normal or nondiagnostic ECGs, IMA levels >93.5 U/mL demonstrated a sensitivity and specificity of 75% for the diagnosis of MI with an area under the ROC curve 0.78. IMA is a Food and Drug Administration approved serum biomarker of cardiac ischemia and a risk stratification tool for acute coronary syndrome, produced during an ischemic condition or attack and is present in the blood in early and easily detectable levels (Peacock et al., 2006).

**Atherogenic index of plasma (AIP)**

Lipid profile consists of a group of biochemical tests often used in predicting, diagnosing and treating lipid related disorders including atherosclerosis (Brites et al., 1998). Generally, the hyperlipidemias are of interest to the physician in the context of risk factors for IHDs (Nwagha et al., 2010). The first step in diagnosis of hyper and hypolipoproteinaemias is to define the lipoprotein pattern by chemical analysis of the plasma lipids and lipoproteins (Burtis and Ashwood, 1996).

Abundant evidence has accumulated relating the concentrations of lipids (total cholesterol and triglycerides) and their associated blood transporting lipoproteins (High density lipoprotein-Cholesterol (HDL-C), Low density lipoprotein-Cholesterol (LDL-C), Very high density lipoprotein-Cholesterol VLDL-C) with the occurrence of atherosclerosis in general and ischemic heart diseases in particular (Cummings, 2003). The strong association between the risk of IHDs, high levels of
**Introduction and Review of Literature**

LDL-C and low levels of HDL-C has been well established (Igweh et al., 2005). However the enormous contributions of triglycerides (TGs) to ischemic risk have been underestimated especially in our environment (Nwagha and Igweh, 2005). Indeed high levels have been associated with an increased incidence of IHD (Hokanson and Austin, 1996) and an increased population of small dense LDL-C particles (Guerin et al., 2001). To estimate the risk of atherosclerosis more accurately the measurement of particle size distribution in LDL has been recommended (Superko, 1996).

Recently Dobiasova and Frohlich (2001) have reported that particle size distribution in both HDL (Dobiasova and Frohlich, 1996) and LDL (Ohta et al., 1997) is reflected in the fractional esterification rate of cholesterol (FER_{HDL}) by lecithin cholesterol acyltransferase (LCAT) in plasma depleted of apolipoprotein (apo) B containing lipoproteins (FER_{HDL}). It has been established that, because of the association of both HDL and LDL particle size with the concentration of plasma TG and HDL cholesterol: the higher TG and the lower HDL-cholesterol levels the smaller the lipoprotein particles and vice versa. LDL and HDL particle sizes are related to plasma levels of triglycerides (TG) (Lamarche et al., 1999).

A lot of work has been done on the relationship between TG and HDL-C and it has been shown that the ratio of TG to HDL-C was a strong predictor of prolonged MI (Gaziano et al., 1997). Universally, atherogenic index of plasma (AIP) calculated as log (TG/HDL-C) has been used as a significant predictor of atherosclerosis (Dobiasova and Frohlich, 2001; Tan et al., 2004).

The measurement of the FER_{HDL} or particle sizes (LDL and HDL) is difficult to put into clinical practice, but it can be easily substituted by calculating the AIP (Dobiasova et al., 2005). As FER_{HDL} (an indirect measure of lipoprotein particle size) also correlates well with plasma concentration of TG's and HDL cholesterol it was hypothesised that it is likely that the ratio of TG/HDL-C will also predict the value of FER_{HDL} and therefore atherogenicity of plasma lipoproteins (Dobiasova and Frohlich, 2001). Thus, the AIP value accurately reflects the presence of atherogenic small LDL particles and small HDL particles and it is also a sensitive predictor of MI risk (Frohlich et al., 2004; Dobiasova et al., 2011).
non-HDL-Cholesterol (non-HDL-C)

non-HDL cholesterol measurement (calculated as total cholesterol minus HDL cholesterol) provides a single index of all the atherogenic, apolipoprotein (apo) B-containing lipoproteins—LDL, VLDL, intermediate density lipoprotein (IDL) and lipoprotein (a). Although apoB can be accessed directly, measurement of non-HDL cholesterol is more practical, reliable and inexpensive and is accepted as a surrogate marker for apolipoprotein B in routine clinical practice. Unlike LDL-C, which can be incorrectly calculated in the presence of postprandial hypertriglyceridemia, non-HDL-C is reliable when measured in the nonfasting state (Grundy, 2002). Just as LDL is the primary carrier of cholesterol in plasma, two remnant lipoproteins—VLDL and IDL—are the main carriers of triglycerides. These triglyceride-rich lipoproteins also carry cholesterol (Chapman and Caslake, 2004).

In the presence of hypertriglyceridemia, triglyceride-rich lipoproteins may be partly depleted of their triglyceride content and become enriched with cholesterol from LDL. The modified remnant lipoproteins that result are believed to be highly atherogenic because of their small size, high cholesterol content and increased residence time in plasma (Marcovina and Packard, 2006). They are able to deliver more cholesterol to macrophages than LDL particles (Krentz, 2003) because they can penetrate the arterial wall with ease, be taken up directly by macrophages and participate in foam cell formation (Lu et al., 2003) thus initiating the lipid laden plaque.

At the same time, LDL exchanges core lipids with VLDL to become TG rich and undergoes lypolysis, resulting in a smaller and denser LDL particle. These compacted, lipid depleted LDL particles are more atherogenic because they are more easily oxidised and readily penetrate the artery wall. However, even though the small, dense LDL particles are greater in both number and atherogeneity than normal sized LDL, LDL cholesterol levels appear normal rather than high on standard measurements because small, dense particles are lipid poor (Marcovina and Packard, 2006). Therefore, the measurement of LDL cholesterol alone does not provide sufficient measure of atherogenic risk (Anne, 2008) in hypertriglyceridemia. Elevated non-HDL cholesterol signifies increased IHD risk, even if LDL cholesterol levels are at or below or appear normal (Grundy, 2002). In clinical trials, non-HDL cholesterol
has been shown to independently predict IHD (Bittner et al., 2002). In the Strong Heart Study, patients with diabetes in the highest tertile of non-HDL cholesterol had a higher hazard ratio for myocardial infarction (Grundy, 2002) than they did with any other lipid parameter (1.96 for LDL cholesterol and 2.04 for triglycerides) compared with those in the lowest tertile. They also had the second highest hazard ratio for coronary heart disease (CHD) (2.75 vs 1.90 for LDL, 2.12 for triglycerides and 3.06 for the total/HDL cholesterol ratio) (Lu et al., 2003).

There is also evidence to suggest that, in patients with diabetes, non-HDL cholesterol is a stronger predictor of mortality from coronary disease than LDL cholesterol. Because non-HDL cholesterol measures the apolipoprotein (apo) B-containing lipoproteins, it can serve as an additional tool to assess cardiac ischemic risk in people whose risk is not accurately identified by LDL cholesterol alone (Anne, 2008). Moreover, non-HDL-C is particularly atherogenic in the presence of the hypertriglyceridemia that usually accompanies MI (Chapman and Caslake, 2004).

**LDL-C/HDL-C**

Controversy exists regarding what is the best method for identifying those who are at increased risk for coronary heart disease (CHD). However, the current National Cholesterol Education Program (NCEP) guidelines recommend specific target levels of LDL and HDL cholesterol for determining IHD risk and evaluating effectiveness of lipid lowering therapies (Maria and Densie, 2008). The number of atherogenic versus non-atherogenic lipoproteins transported in blood provides a more comprehensive evaluation of ischemic heart disease risk. Traditional cholesterol measurements tend to be most accurate at predicting risk for those at the lower and higher ends of the risk spectrum. These measurements are less helpful for the majority of people whose risk falls somewhere in between (Gotto et al., 2000).

The Thirty-Person/Ten-Country Panel recently concluded that the apo B/apo A1 ratio is superior to conventional cholesterol measurements in patients without symptomatic vascular disease or diabetes to evaluate the lipoprotein-related risk of vascular disease (Barter et al., 2006). Both the INTERHEART (Yusuf et al., 2004) and the AMORIS (Walldius et al., 2001) studies show a strong, direct relation between a high apoB/apoA-1 ratio and an increased risk for fatal and acute
myocardial infarction (MI). Moreover, measurements of apo B and apo A-1 tend to reflect the levels of LDL-C and HDL-C (Walldius and Jungner, 2005). A more tenable option that has been proven to be an accurate predictor of cardiovascular risk is the LDL-C/HDL-C ratio, which can be obtained from a standard lipid profile and is more accurate than LDL-C or HDL-C alone (Maria and Densie, 2008). Changes in ratios have been shown to be better indicators of successful IHD risk reduction than changes in absolute levels of lipids or lipoproteins.

Several large epidemiological and clinical studies have found that the LDL-C/HDL-C ratio to be an excellent predictor of CHD risk and an excellent monitor for the effectiveness of lipid lowering therapies (Natarajan et al., 2003; Kannel, 2005). The existing focus on LDL-C as the primary culprit in atherogenesis may divert attention from the more efficient lipid profile of LDL-C/HDL-C. The LDL-C/HDL-C ratio reflects the two way traffic of cholesterol entering and leaving the arterial intima in a way that the individual levels of LDL-C and HDL-C do not (Kannel, 2005). It is evident from the aforementioned literature that LDL-C/HDL-C is a more accurate predictor of risk than LDL-C alone and currently it is the most practical approach available.

**Electrocardiogram: ST segment elevation**

The term ischemic injury was originally derived from the observation that mechanical injury to the heart produces changes of the extracellular electrocardiogram (ECG) closely similar to ischemia. The ECG remains a mainstay in the diagnosis of acute and chronic CHDs. The findings depend upon several key factors including the duration (hyperacute/acute versus evolving/chronic), extent (transmural versus subendocardial) and localisation (anterior versus inferior-posterior) of ischemia or infarction, as well as the presence of other underlying abnormalities. ECG abnormalities were an early sign of MI and could be identified on a ECG within 90 minutes of symptom onset (Kudenchuk et al., 1998).

Persistent and transient ST segment and T or Q wave abnormalities on serial ECGs discriminated those with from whose without acute ischemia or infarction better than changes on a single ECG. Under normal conditions, the ST segment is usually isoelectric (ie, flat along the baseline), because healthy myocardial cells attain
the same potential during repolarisation. Ischemia has complex time-dependent effects on the electrical properties of myocardial cells. Severe, acute ischemia lowers the resting membrane potential and shortens the duration of the action potential in the ischemic area. These changes cause a voltage gradient between normal and ischemic zones, leading to current flow between these regions. These currents of injury are represented on the surface ECG by deviation of the ST segment (Zimetbaum et al., 1998). When acute ischemia is transmural, the ST vector is usually shifted in the direction of the outer (epicardial) layers, producing ST elevations and sometimes tall positive (hyperacute) T waves over the ischemic zone. The shift in the ST vector is due, at least in part, to ischemia-induced shortening of the action potential duration. This pathologic early repolarisation causes the outside surface of ischemic cells to become positively charged relative to nonischemic cells which are still in a depolarised state (negative charge outside). The ECG (in this case, the ST segment) vector always points away from negative and toward positive zones.

When ischemia is confined primarily to the subendocardium, the ST vector typically shifts toward the inner ventricular layer and the ventricular cavity and show ST elevation (Nixdorff et al., 1996). In the past four decades, many investigators have studied the effects of mechanical or ischemic injury on local extracellular electrocardiograms in the heart. Thus, it was gradually established that ST-elevation described in the surface electrocardiogram corresponded in reality to a combination of a TQ-segment change and a real ST-segment change. ST-segment elevation was an indicator of ischemic injury (Andre, 2000). There are a variety of further variables which may be important to the explanation of ischemic ST-segment elevation. Firstly, clinical ischemia may often be related to a limited but not fully interrupted blood supply to the heart. The discrepancy between the sharply demarcated necrotic zone in porcine infarcts and the presence of electrically conducting tissue in human infarcts suggests that the flow pattern in human infarct zones might be complex.

Low flow ischemia cannot be considered as pathophysiologically equivalent to total, no flow ischemia. For example, important ionic changes such as extracellular potassium accumulation are only observed at coronary flow, 30% of normal (Andre, 2000). Furthermore, anoxic perfusion is associated with a considerably larger cellular K⁺ loss than no flow ischemia (Yan et al., 1993). Thus, relatively small changes in
flow reduction are likely to have an impact on the ionic and the associated electrical changes. Secondly, clinical ST-segment elevation after myocardial infarction often persists after the acute phase of ischemia, especially in the case of ventricular aneurysm. Persistent ischemic damage in the border zone of myocardial infarction, combined with a low electrical impedance of scar tissue in the center of the infarction may partially explain this phenomenon (Cinca et al., 1998; Cinca et al., 1995). As a third factor affecting ST-segment elevation, the influence of the autonomous nervous system should be mentioned. Increased sympathetic tone affects the amount of ST-segment elevation observed early in ischemia and metabolism related depletion of noradrenaline stores in ischemic myocardium importantly contributes to the electrophysiological changes observed after coronary occlusion (Andre, 2000).

**Myocardial metabolism under normoxic condition**

Before considering myocardial metabolism during ischemia, it is important to have an understanding of metabolism in the normal healthy heart (Figure 1). The pump function of cardiac muscle is supported by high rates of myocardial blood flow, oxygen consumption and combustion of fat and carbohydrates.

![Figure 1: Myocardial metabolism under normoxic condition.](Tomohisa et al., 2011)
ANT, adenine nucleotide translocase; ACC, acetyl-CoA carboxylase; CPT, carnitine palmitoyltransferase; FABP, fatty acid binding protein; FACS, fatty acyl-CoA synthase; FAT, fatty acid transporter; FFA, free fatty acid; GLUT, glucose transporter; G-6-P, glucose-6-phosphate; MCD, malonyl-CoA decarboxylase; PFK, Phosphofructokinase; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid cycle.

Myocardial ischemia occurs when there is a deficit between the normal rate of oxygen delivery to the myocardium required for a given heart rate, after load and inotropic state and the actual rate of oxygen delivery to the myocardium. The primary effect of ischemia is mitochondrial metabolic dysfunction caused by reduced aerobic formation of adenosine triphosphate (ATP), this triggers accelerated anaerobic glycolysis and disruption of normal cardiac cell function (Opie, 1998).

**Cardiac function: maintained by adenosine triphosphate**

Under conditions of normal coronary blood flow, ATP is broken down by myosin ATPase, releasing energy that fuels tension development and systolic work. ATP breakdown is also employed by the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase to remove Ca\(^{2+}\) from the cytosol at the end of systole and allow for diastolic relaxation (Stanley et al., 1997). In the healthy heart the processes of ATP synthesis and breakdown are exquisitely matched such that there is never a significant fall in ATP concentration, even with large increases in cardiac power output (Stanley, 2001). The main energy producing mechanism for ATP synthesis is oxidative phosphorylation in mitochondria. This process is maintained by the production of reducing equivalents (i.e. NADH) mainly through the tricarboxylic acid cycle from controlled combustion of the substrates, free fatty acids (FFA) and carbohydrates (glucose, lactate, pyruvate). The combined functions of the respiratory chain with the intervention of oxygen and ATPase (ATP synthase) allow the repohosphorylation of ADP to ATP.

Adenine nucleotide translocase (ANT) controls the exchange of ATP and ADP between the mitochondrial matrix and cytosol. Several creatine kinases (CK) participate in the transfer of energy between ATP and phosphocreatine (PCr) (Truls, 2008). The presence of CK specifically bound to mitochondria and to myofibrils creates a shuttle of energy from mitochondria to the sites of ATP utilisation e.g. myofibrils and ion pumps. The main site of ATP dephosphorylation is the myofibrillar ATPase, but other ATPases associated with different membranes (Na\(^+\)/K\(^+\)-ATPase, Ca\(^{2+}\)-ATPase, etc.) also participate in the expenditure and cleavage of ATP. Several
intracellular compounds are proposed to play a role in the regulation of mitochondrial ATP production. These are phosphorylated compounds (ADP), redox state (NADH) or calcium (Ca\(^{2+}\)). The production of ATP is also dependent on oxygen (O\(_2\)) supply. The main storage form of high energy phosphate is phosphorylated creatine (PCr). Cr and PCr are smaller and less negatively charged than ATP and ADP. Consequently, they can be stored in much higher concentrations and can be more easily transported to the different sites in the cell (Wyss and Kaddurah, 2000).

**Glucose and lactate metabolism**

Glucose and lactate supply between approximately 10% and 40% of the energy requirement of the heart (Wisneski et al., 1990). Glucose is taken up by the myocardium and is either stored as glycogen, or broken down by glycolysis to pyruvate in the cytosol of the cell. Lactate is extracted from the blood, converted to pyruvate in the cytosol and further oxidised to acetyl-CoA in the mitochondrial matrix. In the normal healthy human heart, pyruvate is derived in approximately equal proportions from glycolysis and lactate uptake (Stanley, 2001). Pyruvate is oxidised to acetyl-CoA in the mitochondria by the enzyme pyruvate dehydrogenase (PDH). The rate of flux of pyruvate to acetyl-CoA is determined by the amount of active enzyme present in the tissue and the concentration of the substrates (CoA, nicotinamide adenine dinucleotide (NAD\(^+\)) and pyruvate) and the products (acetyl-CoA and NADH) (Stanley et al., 1997).

**Fatty acids are the predominant fuel for the heart**

Fatty acids supply approximately 60–90% of the energy used to synthesize ATP in the healthy human heart (Wisneski et al., 1990). The rate of fatty acid uptake by the heart is primarily determined by the concentration of fatty acids in the plasma, which varies widely between 0.1 and approximately 1.5 mmol/L. Plasma fatty acids come from the breakdown of triglyceride in fat cells and the broad range in plasma concentration is due to the hormonal control of hormone sensitive lipase by insulin and noradrenaline (norepinephrine) in this tissue. Insulin suppresses fatty acid levels and thus fatty acid levels are low when insulin levels are high after a meal. On the other hand, noradrenaline increases fatty acid release from fat cells so that fatty acid levels are elevated under times of stress, such as physical exercise, fasting or myocardial ischemia.
Thus, during times of stress, when catecholamines are high and insulin is low, the heart is faced with a high plasma free fatty acid concentration and fatty acid oxidation by the heart is high (Stanley, 2001). Fatty acids are oxidised in the mitochondria where they release energy in the form of reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH₂) for the electron transport chain and the subsequent formation of ATP by oxidative phosphorylation (Lopaschuk et al., 1994).

On entering the cell, fatty acids are esterified to fatty acyl-coenzyme A (CoA), which makes the fatty acid more water soluble. In order to cross the inner mitochondrial membrane, the fatty acid must be converted to fatty acylcarnitine by the enzyme carnitine palmitoyl transferase (Stanley, 2001). Once in the mitochondrion the fatty acid undergoes beta-oxidation, a process that repeatedly cleaves off two carbon acetyl-CoA units, generating NADH and FADH₂ in the process. The acetyl-CoA is further oxidized to CO₂ in the citric acid cycle. The rate of fatty acid beta-oxidation is primarily regulated by the concentration of free fatty acids in the plasma, the activity of the carnitine transferase/translocase system on the mitochondrial membranes and the activity of a series of enzymes that catalyse the multiple steps of fatty acid beta-oxidation (Lopaschuk et al., 1994).

Cardiac metabolism during ischemia

There is compelling evidence, from both clinical and experimental studies that the failing heart is characterised by disturbances in the myocardial metabolism (Shen et al., 1999; Ingwall and Shen, 1999).

Glycolysis and lactate production

The primary result of ischemia is mitochondrial metabolic dysfunction caused by reduced oxygen delivery to the tissue, resulting in a decrease in ATP formation by oxidative phosphorylation (Opie, 1998). The reduction in aerobic ATP formation stimulates glycolysis and an increase in myocardial glucose uptake and glycogen breakdown occurs (Stanley et al., 1997). Unlike under conditions of normal blood flow, however, during ischemia pyruvate produced by glycolysis is not so readily oxidised in the mitochondria and there is a high rate of conversion of pyruvate to lactate in the cytosol and a rise in tissue lactate content. Instead of the normal uptake
Introduction and Review of Literature

of lactate from the blood, the ischemic myocardium switches to production of lactate. Cell homeostasis is dramatically disrupted, there is accumulation of lactate and H⁺, a fall in intracellular pH and a reduction in contractile work. Thus, during ischemia there is accelerated glycolysis and pyruvate formation concurrent with impaired pyruvate oxidation in the mitochondria, which results in lactate accumulation in the tissue. The ischemia induced fall in intracellular pH has several negative effects on the ability of cardiac muscle to maintain Ca²⁺ homeostasis and use the energy released from the that occurs in exchange for Na⁺ leads to a greater Na⁺–Ca²⁺ exchange across the cell membrane and further wasting of ATP in order to maintain Ca²⁺ homeostasis (Murphy et al., 1991). Figure 2 depicts the cardiac energy metabolism under ischemic conditions.

Figure 2: Cardiac energy metabolism during ischemia

The up and down arrows indicate the changes compared with normal aerobic conditions. Relative to the aerobic conditions, ischemia results in an increase in glycolysis without an increase in the rate of pyruvate oxidation, thus causing lactate to accumulate in the cell. Despite accelerated glycolysis and lactate production, the relatively high rate of residual oxygen consumption is fuelled primarily by the oxidation of fatty acids. ADP=adenosine diphosphate; ATP=adenosine triphosphate; Pi=inorganic phosphate.

Perturbations of myocardial lipid metabolism

Alterations in myocardial lipid metabolism during ischemia have been implicated as biochemical mechanisms that mediate the responses to ischemia. These responses include mechanisms involved in the pathophysiological sequelae of ischemic and post
ischemic myocardium. In general, alterations in myocardial lipid metabolism during ischemia can be classified into two groups:

1) Changes in fatty acid β-oxidation and

2) Changes mediated by the activation of phospholipases and other lipid catabolic enzymes that target the structurally important lipid constituents of cellular membrane structures of the heart (Chen and Gross, 1994).

Lipid metabolism during MI is complex. There are multiple enzymes that coordinate the catabolism of fatty acids and the degradation of membrane lipids during ischemia within different subcellular organelles and membrane pools. Alterations in myocardial lipid metabolism during MI can have profound effects on the physiological status of the heart through changes in membrane fluidity, membrane integrity and the activation of signaling cascades (Pak et al., 1987; Chen and Gross, 1994).

Fatty acids are the main fuel for the mitochondria under ischemia

The heart derives as much as 60-90% of its energy needs from the catabolism of fatty acids. During moderate MI, the residual oxygen consumption is largely supported by the oxidation of fatty acids (Lopaschuk et al., 1994a). In fact, the relative contribution of fatty acids to the energy requirement of the myocardium is not significantly affected by ischemia of moderate severity (McNulty et al., 1996). Studies conducted in large animal models show that reductions in coronary blood flow of 30–60% do not affect the relative contribution of fatty acids to mitochondrial oxygen consumption, despite a dramatic switch to lactate production.

In studies conducted in swine and dogs using isotopically labelled glucose and fatty acid tracers, there was a continued high rate of fatty acid oxidation with an increase in the relative contribution of glucose to mitochondrial oxidative metabolism when coronary blood flow was reduced by 30–60% (Stanley, 2001). Even though there was a switch from lactate uptake to lactate production and a decrease in myocardial ATP content during ischemia, fatty acid continued to be the predominant fuel for the heart. It is important to note that patients undergoing MI have very high plasma free fatty acid concentrations (>1.0 mmol/L) because of activation of the peripheral sympathetic nervous system (Lopaschuk et al., 1994b), which would fuel a high relative contribution of fatty acid to myocardial substrate oxidation. During MI,
depressed oxygen supply results in the uncoupling of oxidative phosphorylation, leading to the inhibition of fatty acid β-oxidation as reducing equivalents become limiting. As a result, fatty acid metabolic intermediates increase during MI. Most notably, acyl carnitine, β-hydroxy fatty acid intermediates and acyl CoA molecular species accumulate during MI due to the inhibition of β-oxidation. The accumulation of fatty acids and their intermediates during MI also has been shown to be deleterious (Wu et al., 1993). Several biochemical mechanisms are possible that could lead to apoptosis in the ischemic heart in the presence of fatty acids. One mechanism that has been proposed for palmitate induced apoptosis involves the accumulation of palmitoyl CoA, which is the precursor, along with serine, for the synthesis of sphingosine and ceramide (a proapoptotic lipid) (Obeid et al., 1993). Another mechanism through which alterations in fatty acid metabolism in the ischemic heart may elicit myocardial apoptosis is through the accumulation of palmitoylcarnitine. Palmitoylcarnitine has been shown to activate the proapoptotic caspases (Mutomba et al., 2000).

**Cardiac lipotoxicity**

Cardiomyocytes, however, like other nonadipose organs, have very limited capacity for storage of lipids. When this capacity is exceeded, the resultant process of cellular dysfunction or cell death is termed lipotoxicity (Unger, 2002). Lipid accumulation in nonadipose tissues occurs in disease states with an excess availability of plasma FA and triglycerides (TG) causing a mismatch between lipid uptake and utilisation (Kankaanpaa et al., 2006; McGavock et al., 2007). In MI additional sources of lipids contribute to lipid accumulation besides the extracellular lipids. The fact that different lipid moieties accumulate in ischemic hearts in experimental settings, without lipids in the perfused medium indicate that intracellular endogenous esterified fatty acid pools, such as membrane phospholipids, contribute to the rise of lipids in ischemic cardiac tissue (Truls, 2008).

Early studies have shown that morphologically distinctive feature of ischemic myocardium is the formation of lipid droplets. Lipid droplets within the heart are comprised of a mixture of cholesterol esters and triglycerides, which are positive to oil red-O staining and are observed in ischemic myocardial tissue (Straeter et al., 1996). The accumulation of these droplets in the ischemic heart is likely due to β-oxidation inhibition. Similar to the mismatch of fatty acid import with oxidation that
occurs during MI, a transgenic mouse line overexpressing acyl CoA synthase has been shown to have a mismatch of intracellular fatty acid import with utilisation leading to lipid droplet formation (Chiu et al., 2001).

In contrast to the potential effects of lipid deposition on ischemic myocardial function, it has also been proposed that acylcarnitine molecular species are arrhythmogenic and contribute to the sudden death associated with MI (Ford, 2002). Inhibition of β-oxidation during myocardial ischemia leads to the accumulation of acyl CoA and acylcarnitine in the myocardium. Acyl CoA, due to its polar nature, remains largely within the mitochondrial matrix, whereas acylcarnitine can diffuse in and out of the cell due to its amphiphilic properties.

The detrimental effects of myocardial lipid accumulation during tissue ischemia and reperfusion have been recognised for a long time. The concept of lipotoxicity has gained renewed appreciation in recent years. The adverse effects of myocardial lipid overload are well documented in different animal models. A commonly used model of fatty heart is the Zucker diabetic fatty (ZDF) rat. In the ZDF rat, a loss of function mutation in the leptin receptor (Iida et al., 1996) in the hypothalamic centers that regulate feeding behavior results in increased food intake, whereas in peripheral tissues, such as the pancreatic islets, it results in markedly increased lipogenesis. Consequently, the combination of increased caloric influx and a generalised increase in lipogenesis in tissues causes an accelerated steatosis in cardiomyocytes and other organs. Steatosis of the myocardium is associated with left ventricular hypertrophy and dysfunction that ultimately progresses to lipotoxic cardiomyopathy (Zhou et al., 2000). Several transgenic animal models of lipotoxic cardiomyopathy have been created.

If hearts internalise excess lipid or have a defect in lipid oxidation, then lipid storage must increase. Augmentation of lipid uptake has been achieved through transgenic expression of a cell membrane anchored form of lipoprotein lipase (LpL) (Yagyu et al., 2003), overexpression of (MHC)-long-chain acyl coenzyme A synthetase (ACS)1 (Chiu et al., 2001), MHC-fatty acid transport protein (FATP)1 (Chiu et al., 2005) and transgenic expression of PPARγ (Son et al., 2007). The lipid accumulation in these models is associated with the development of various degrees
of cardiomyopathy with left ventricular (LV) hypertrophy and dilatation, depressed cardiac systolic and/or diastolic function and with premature death in some models. Transgenic mouse models with specific defects in the mitochondrial fatty acid oxidation pathways have also been established (Watanabe et al., 2000). These models all exhibit cardiac lipotoxicity, although, these models display disparate cardiomyopathic phenotypes. For example, deletion of a fatty acid chain length specific dehydrogenase enzyme (VLCAD) shows increased susceptibility to ventricular tachycardia and arrhythmias without overt systolic dysfunction (Exil et al., 2003). Myocardial lipid accumulation is present in many patients with inherited defects in fatty acid oxidative enzymes die suddenly, suggests that lipotoxicity may precipitate sudden myocardial dysfunction (Mathur et al., 1999). This suggests that not only lipid accumulation per see but also the profile of the accumulating lipid moieties are important. Lysophosphatidylcholine (LPC), a hydrolysis product of phospholipid degradation by action of the phospholipases, accumulates in ischemic myocardium and this accumulation has been associated alterations in the action potential resembling those observed in ischemic myocardium in vivo (Truls, 2008).

Other proposed mechanisms responsible for the toxicity of accumulating lipids are several: direct toxic effects of neutral droplets or fatty acids on myofibrillar function (Dyntar et al., 2001), activation of apoptotic signaling pathways (Pettus et al., 2002), reactive oxygen species generated as a toxic byproduct of lipid oxidation (Schrauwen and Hesselink, 2004), mitochondrial dysfunction (Ostrander et al., 2001), disturbed calcium handling (Korge et al., 2003), nitric oxide generation (Truls, 2008). Most data indicate that the TGs themselves serve primarily a storage function with toxicity deriving mainly from non-esterified fatty acids (NEFA) and their products such as ceramides, diacylglycerols and CoA and carnitine esters of fatty acid (FA) that accumulate either as a result of failure of esterification or breakdown of the triglycerides (Listenberger et al., 2003). Pathophysiological states such as MI, heart failure and cardiac hypertrophy are associated with myocardial accumulation of different lipid moieties in the heart (Barger et al., 2000; Sharma et al., 2004).

**Pyruvate oxidation inhibition during ischemia**

The impaired pyruvate oxidation during ischemia of moderate severity is due to the rise in mitochondrial NADH secondary to the fall in oxygen consumption and
to the high rates of fatty acid oxidation (Figure 3). During this, there is a buildup of NADH and a rise in the NADH:NAD$^+$ ratio in the mitochondria, which feedback and inhibit flux through PDH via product inhibition. The build-up of NADH during ischemia is the result of the decrease in oxygen consumption and electron transport chain flux, resulting in a back-up of NADH oxidation and a fall in NAD$^+$ content. Studies conducted in pigs and dogs showed that ischemia does not decrease the degree of direct PDH inhibition by phosphorylation (Schoder et al., 1998), suggesting that the impairment in the in vivo rate of pyruvate oxidation during ischemia is not due to deactivation of the enzyme, but rather to product inhibition by an increase in the NADH:NAD$^+$ ratio.

**Figure 3: Pyruvate oxidation during ischemia.**

There is accelerated glycolysis and lactate production in the cytosol. In the mitochondria there is a rise in the ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD$^+$) due to a decrease in oxygen consumption and continued fatty acid oxidation.

**Phospholipid catabolism during ischemia**

The subcellular membrane pools of the heart are comprised of a diverse array of phospholipid classes, subclasses and molecular species, which provide multiple specific substrates for the multitude of lipolytic enzymes within the heart cell. The result of the catabolism of cardiac phospholipids is the production of many types of lipidic second messengers that likely mediate changes in the function of important intracellular proteins as well as the signaling of nuclear transcription factors that
dictate the future physiologic state of the cells. Under pathophysiological conditions of severe ischemia, it is possible that the unregulated activity of the lipolytic enzymes may propagate irreversible damage to the heart. For example, the discontinuities of the sarcolemma of the heart seen in irreversibly damaged myocardium following severe ischemia has been considered to be mediated in part by the activation of phospholipases that digest the sarcolemma membrane (Ford, 2002).

Free radical and oxidant modifications of phospholipids

Many studies have implicated the production of oxygen free radicals as mediators of myocardial injury in the ischemic heart. Electron spin resonance studies have demonstrated that ischemia results in the appearance of oxygen free radicals with the concomitant appearance of lipid peroxidation products such as conjugated dienes (Kramer et al., 1994). With the production of fatty acids produced by phospholipases and the activation of protein kinase C during ischemia, the production of lipid derived products of oxidation has dual role the production of lipid derived free radicals and are deleterious to the heart (Starkopf et al., 1998). Another potential mechanism for the oxidation of cardiac lipids during ischemia is possibly mediated by neutrophils. It is well established that activated neutrophils release both myeloperoxidase and hydrogen peroxide that together in the presence of physiological levels of sodium chloride, produce hypochlorous acid (Ford, 2002).

Free radicals

Oxygen a necessary component of living organisms, may at times produce reactive species known as oxygen free radicals which are detrimental to living tissue. Aerobic organisms use oxygen to oxidize carbon and hydrogen rich substrates to obtain energy essential for life. When organic molecules are oxidised, oxygen is reduced to form water by concerted four electron transfer. However, oxygen can also undergo univalent reduction by one electron transfer allowing the formation of oxygen radicals and other oxygen reactive species. A free radical is defined as a molecule or ion containing an unpaired electron at its outer orbital which renders it highly reactive (Evans and Halliwell, 2001; Gilbert, 2000).

Free radicals include hydroxyl (OH·), superoxide (O₂⁻), nitric oxide (NO·), nitrogen dioxide (NO₂·), peroxyl (ROO·) and lipid peroxyl (LOO·). Also, hydrogen
peroxide (H₂O₂), ozone (O₃), singlet oxygen (¹O₂), hypochlorous acid (HOCI), nitrous acid (HNO₂), peroxynitrite (ONOO⁻), dinitrogen trioxide (N₂O₃), lipid peroxide (LOOH), are not free radicals and generally called oxidants, but can easily lead to free radical reactions in living organisms (Genestra, 2007). Reactive oxygen species (ROS) and reactive oxygen species (RNS) are the terms collectively describing free radicals. Formation of ROS and RNS can occur in the cells by enzymatic and non-enzymatic reactions. Enzymatic reactions include respiratory chain, phagocytosis, prostaglandin synthesis and the cytochrome P450 system (Pham-Huy et al, 2008; Valko et al., 2007). For example, the superoxide anion radical (O₂⁻) is generated via several cellular oxidase systems such as NADPH oxidase, xanthine oxidase, peroxidases. Once formed, it participates in several reactions yielding various ROS and RNS such as hydrogen peroxide, hydroxyl radical (OH⁻), peroxynitrite (ONOO⁻), hypochlorous acid (HOCI), etc. H₂O₂ (a non radical) is produced by the action of several oxidase enzymes, including aminoacid oxidase and xanthine oxidase. The last one catalyses the oxidation of hypoxanthine to xanthine and of xanthine to uric acid.

Super oxide (O₂⁻)

The result of monovalant reduction of triplet oxygen is called super oxide, abbreviated as (O₂⁻). Super oxide is a radical; it is usually shown with a negative sign, indicating that it carries a negative charge of -1.

\[ {}^\cdot\text{O} \rightarrow {}^\cdot\text{O}^- \quad \text{Triplet oxygen (Ground state)} \]
\[ \quad \text{monovalant reduction} \]
\[ {}^\cdot\text{O} \rightarrow {}_\text{O}^- \quad \text{O: Super oxide} \]

Super oxide anion is the first reduction product of oxygen (O₂). It can be produced either by the univalent reduction of O₂ or by the univalent oxidation of H₂O₂. However, super oxide is not particularly reactive in biological system and does not by itself cause much oxidative damage. It is a precursor to other oxidizing agents, including singlet oxygen, peroxynitrite and other highly reactive molecules (Jan, 2010). The biological toxicity of super oxide is due to its capacity to inactivate iron sulfur cluster containing enzymes (which are critical in a wide variety of metabolic pathways), thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. In its HO₂⁻ form, super
Introduction and Review of Literature

Oxide can also initiate lipid peroxidation of polyunsaturated fatty acids. It also reacts with carbonyl compounds and halogenated carbons to create toxic peroxy radicals (Wan et al., 1994). Super oxide can also react with nitric oxide (NO) to form ONOO\(^-\). As such, super oxide is one of the main causes of oxidative stress (SOX). Super oxide donates one electron to reduce the metal ions that acts as the catalyst to convert hydrogen peroxide into hydroxyl radical (OH\(^-\)).

\[ \text{O}_2^+ + \text{Fe}^{3+} \rightarrow 3\text{O}_2 + \text{Fe}^{2+} \]

The reduced metal (ferrous ion or Fe\(^{2+}\)) then catalyses the breaking of the hydrogen-oxygen bond of hydrogen peroxide to produce a hydroxyl radical (\(^\cdot\text{OH}\)) and a hydroxyl ion (OH\(^-\)).

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^+ + \text{OH}^- \]

Super oxide can react with the hydroxyl radical to form singlet oxygen (\(^1\text{O}_2\)) which is not a radical but reactive nonetheless (Halliwell and Gutteridge, 1999).

\[ \text{O}_2^+ + \text{OH} \rightarrow ^1\text{O}_2 + \text{OH}^- \]

Hydroxyl Radical (OH\(^-\))

The hydroxyl radical, \(^\cdot\text{OH}\), is the neutral form of the hydroxide ion. Hydroxyl radicals are highly reactive and consequently short lived; however, they form an important part of radical chemistry. Most notably hydroxyl radicals are produced from the decomposition of hydroperoxides (ROOH) (Bielski and Cabelli, 1995). Hydrogen peroxide in the presence of metal ions (Cu\(^+\)/Fe\(^{2+}\)), is converted to a hydroxyl radical (\(^\cdot\text{OH}\)) and hydroxide ion (OH\(^-\)).

The metal ion is required for the breaking of the oxygen-oxygen bond of peroxide. This reaction is called the “Fenton Reaction” and was discovered over a hundred years ago. It is important in biological systems because most cells have some level of iron, copper, of other metals which can catalyze this reaction (Valko et al., 2005; Leonard et al., 2004).

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \text{ (Fenton reaction)} \]

\[ \text{O}_2^+ + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}^- \text{ (Haber-Weis reaction)} \]
Introduction and Review of Literature

The hydroxyl radical has a very short in vivo half-life of approximately $10^{-9}$ seconds and high reactivity. This makes it a very dangerous compound to the organism (Pastor et al., 2000). Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction, as this would require its diffusion to the enzyme's active site. As diffusion is slower than the half-life of the molecule, it will react with any oxidizable compound in its vicinity. It can damage virtually all types of macromolecules: carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (Ron and Abraham, 2002).

Hydrogen Peroxide ($\text{H}_2\text{O}_2$)

Hydrogen peroxide is the most stable reactive oxygen metabolite (ROMs). This is to say that it is the least reactive and the most readily detected. $\text{H}_2\text{O}_2$ may be generated directly by divalent reduction of $\text{O}_2$ or indirectly by univalent reduction of superoxide anion. $\text{H}_2\text{O}_2$ is the primary product of the reduction of $\text{O}_2$ by numerous oxidases such as xanthine oxidase (XO), uricase, localized in peroxisomes (Ray and Husain, 2002). Hydrogen peroxide can be generated from the two electron reduction of oxygen. In biological systems hydrogen peroxide is generated by the production of super oxide: two super oxide molecules can react together to form hydrogen peroxide and oxygen (Halliwell and Gutteridge, 1999).

$$2\text{O}_2 + 2\text{H} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

The above reaction is called a dismutation reaction as the radical reactants produce nonradical products. Although hydrogen peroxide ($\text{H}_2\text{O}_2$) is a non-radical form of ROS and only possesses moderate oxidant reactivity, it is probably more harmful than $\text{O}_2^-$, because $\text{H}_2\text{O}_2$ can easily diffuse across plasma membrane, enter the inner compartments of cell and can directly damage DNA, lipids and other macromolecules causing oxidative injury to the cell (Halliwell et al., 2000). When not metabolized, $\text{H}_2\text{O}_2$ can react with partially reduced transition metals such as $\text{Fe}^{2+}$ or $\text{Cu}^+$, resulting in the generation of the extremely reactive hydroxyl radical ('OH) that will lead to the propagation of the oxidative damage to the cell (Sandstrom, 1991).

Singlet Oxygen ($^1\text{O}_2$)

It is a nonradical (does not have an unpaired electron) reactive oxygen species often associated with oxygen free radicals that has strong oxidising activity. Singlet
Introduction and Review of Literature

Oxygen (\(^1\text{O}_2\)) is an electronically excited and mutagenic form of oxygen. It is generated by input of energy like radiation, but can also be generated enzymatically by the action of peroxidases or lipoxigenases or by the reaction of \(\text{H}_2\text{O}_2\) with hypochlorite or peroxynitrite (DiMascio et al., 1994). They are also generated in biological systems in a number of pigment reactions including chlorophylls, retinal and flavins when they are illuminated in the presence of oxygen. Like many other reactive species, this can be harmful at higher concentrations and at low levels may act as signaling molecules. Due to its relatively long life, \(^1\text{O}_2\) can travel appreciable distance in the cellular environment and is capable of damaging various biomolecules (Sies, 1993). Oxidative damage in biomolecules mediated by \(^1\text{O}_2\) is rather frequent. Lipids, proteins and DNA are all at risk (Devasagayam and Kamat, 2002).

Peroxynitrite

Peroxynitrite is the anion with the formula ONOO\(^{-}\). It is an unstable "valence isomer" of nitrate, \(\text{NO}_3^-\), which has the same formula but a different structure. Although peroxynitrous acid is highly reactive, its conjugate base peroxynitrite is stable in basic solution (Holleman and Wiberg, 2001). It is prepared by the reaction of hydrogen peroxide with nitrite.

\[
\text{H}_2\text{O}_2 + \text{NO}_2^- \rightarrow \text{ONOO}^- + \text{H}_2\text{O}
\]

Peroxynitrite is an oxidant and nitrating agent. Because of its oxidizing properties, peroxynitrite can damage a wide array of molecules in cells, including DNA and proteins. Formation of peroxynitrite in vivo has been ascribed to the reaction of the free radical super oxide with the free radical nitric oxide (Pacher et al., 2007).

\[
\text{O}_2^- + \text{NO} \rightarrow \text{ONO}_2^-
\]

The resultant pairing of these two free radicals results in peroxynitrite, a molecule which itself is not a free radical, but is a powerful oxidant.

Nitrite oxide (NO\(^{\cdot}\))

NO\(^{\cdot}\) is considered as a free radical with limited reactivity but it can react with \(\text{O}_2\), \(\text{O}_2^-\) and transition metals to form more powerful oxidant (Markesbery and Carney, 1999). NO\(^{\cdot}\) is endogenously produced and initially characterised as endothelial derived relaxing factor. NO\(^{\cdot}\) is now found to be involved in biological actions ranging
from vasodilation, neurotransmission, inhibition of platelet adherence and aggregation and macrophage and neutrophil mediated killing of pathogens (Moncada et al., 1991). It is synthesised from L-arginine in a variety of cells and tissues by nitric oxide synthase (NOS) (Zamora and Billiar, 2000). Three isoforms of NOS account for NO production including neuronal NOS (nNOS; type I) which originally identified as constitutive in neuronal tissue, inducible NOS (iNOS; type II) which is originally identified as being inducible by cytokines in activated macrophages and liver and endothelial NOS (eNOS; type III) which is originally identified as constitutive in vascular endothelial cells (Fang et al., 2002). Production of NO in the central nervous system by nNOS accounts for most of NO activity (Sasaki et al., 2000). NO is produced excessively in excitotoxicity, inflammation and ischemic injury (Bredt and Snyder, 1994). High concentrations of NO are toxic and interact with O2• to form peroxynitrite (Snyder and Bredt, 1991).

Significance of free radicals

Free radicals can be produced from non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. The non-enzymatic process can also occur during oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria (Ganestra, 2007; Valko et al., 2007; Droge, 2002). ROS and RNS are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from ischemia, immune cell activation, inflammation, mental stress, excessive exercise, infection, cancer, aging. Exogenous ROS/RNS result from cigarette smoke, air and water pollution, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat), radiation (Pacher et al., 2007; Willcox et al., 2004; Young and Woodside, 2001; Parthasarathy et al., 1999).

After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals. But it has to be emphasised that ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. At low or moderate concentrations, ROS and RNS are necessary for the maturation process of cellular structures and act as
Introdition and Review of Literature

weapons for NO on demand. Hence, it is worth emphasising the important beneficial role of free radicals (Devasagayam et al., 2004).

* Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation.
* Detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes).
* Apoptosis of effete or defective cell.
* Killing of microorganisms and cancer cells by macrophages and cytotoxic lymphocytes.
* Oxygenases (eg.COX: cyclooxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions (Devasagayam et al., 2004).

Other beneficial effects of ROS and RNS involve their physiological roles in the function of a number of cellular signaling systems (Pacher et al., 2007). Their production by nonphagocytic NADPH oxidase isoforms plays a key role in the regulation of intracellular signaling cascades in various types of nonphagocytic cells including cardiac myocytes, fibroblasts, endothelial cells, vascular smooth muscle cells and thyroid tissue.

For example, NO is an intercellular messenger for modulating blood flow, thrombosis and neural activity (Pham-Huy et al., 2008). NO is also important for nonspecific host defense and for killing intracellular pathogens and tumors. Another beneficial activity of free radicals is the induction of a mitogenic response (Genestra, 2007). In brief, ROS/RNS at low or moderate levels are vital to human health. When produced in excess, free radicals and oxidants generate a phenomenon called SOX, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins and deoxyribonucleic acid (DNA) (Valko et al., 2006; Valko et al., 2005).

Hydroxyl radical and peroxynitrite in excess can damage cell membranes and lipoproteins by a process called lipid peroxidation. This reaction leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. Lipid peroxidation occurs by a radical chain reaction, i.e. once started, it spreads rapidly and affects a great number of lipid molecules (Frei, 1997). Proteins may also be damaged by ROS/RNS, leading to structural changes and
loss of enzyme activity (Halliwell, 2007). The damage to DNA leads to the formation of different oxidative DNA lesions which can cause mutations. The body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants (Willcox et al., 2004). Figure 4, depicts the major sources for free radicals and their consequences of free radical damage.

Figure 4: Major sources of free radicals in the body and the consequences of free radical damage.

Antioxidants

Cells have developed a comprehensive set of antioxidant defense mechanisms to limit the action of ROS. Under physiological conditions, ROS generation is controlled by a large number of antioxidants. Antioxidants with the property to scavenge and detoxify oxidative agents act antagonistically. An antioxidant system is defined as any substance that can inhibit or delay the oxidation of various molecules; it involves some enzymatic components such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and nonenzymatic low molecular weight components viz reduced glutathione (GSH), albumin, bilirubin, uric acid (UA), selenium, vitamins C, E and carotenoids (Sorg, 2004).
Superoxide Dismutase (SOD)

The superoxide dismutases catalyse the dismutation of superoxide to hydrogen peroxide:

\[ O_2^{-} + O_2^{-} + 2H^+ \rightarrow H_2O_2 + O_2 \]

The hydrogen peroxide must then be removed by CAT or GPx, as described above. There are three forms of SOD in mammalian tissues, each with a specific subcellular location and different tissue distribution.

• Copper zinc superoxide dismutase (CuZn-SOD): CuZnSOD is found in the cytoplasm and organelles of virtually all mammalian cells (Liou et al., 1993). It has a molecular mass of approximately 32 000 kDa and has two protein subunits, each containing a catalytically active copper and zinc atom.

• Manganese superoxide dismutase (MnSOD): MnSOD is found in the mitochondria of almost all cells and has a molecular mass of 40 000 kDa (Young and Woodside, 2001). It consists of four protein subunits, each probably containing a single manganese atom. The amino acid sequence of MnSOD is entirely dissimilar to that of CuZnSOD and it is not inhibited by cyanide, allowing MnSOD activity to be distinguished from that of CuZnSOD in mixtures of the two enzymes.

• Extracellular superoxide dismutase (EC-SOD): EC-SOD was described by Marklund (1982) and is a secretory copper and zinc containing SOD distinct from the CuZnSOD described above. EC-SOD is synthesised by only a few cell types, including fibroblasts and endothelial cells and is expressed on the cell surface where it is bound to heparan sulphates. EC-SOD is the major SOD detectable in extracellular fluids and is released into the circulation from the surface of vascular endothelium following the injection of heparin (Karlsson et al., 1993). EC-SOD might play a role in the regulation of vascular tone, because endothelial derived relaxing factor (nitric oxide or a closely related compound) is neutralised in the plasma by superoxide (McIntyre et al., 1999).

Catalase (CAT)

Catalase was the first antioxidant enzyme to be characterised and catalyses the two stage conversion of hydrogen peroxide to water and oxygen:

\[ \text{Catalase} - \text{Fe (III)} + H_2O_2 \rightarrow \text{compound I} \]

\[ \text{Compound I} + H_2O_2 \rightarrow \text{catalase-Fe (III)} + 2H_2O + O_2 \]
Catalase consists of four protein subunits, each containing a haem group and a molecule of NADPH (Young and Woodside, 2001). The rate constant for the reactions described above is extremely high (~107 M/sec), implying that it is virtually impossible to saturate the enzyme in vivo. Catalase is largely located within cells in peroxisomes, which also contain most of the enzymes capable of generating hydrogen peroxide. The amount of catalase in cytoplasm and other subcellular compartments remains unclear, because peroxisomes are easily ruptured during the manipulation of cells. The greatest activity is present in liver and erythrocytes but some catalase is found in all tissues.

Glutathione peroxidases and glutathione reductase (GPx and GR)

Glutathione peroxidases catalyse the oxidation of glutathione at the expense of a H₂O₂, which might be H₂O₂ or another species such as a lipid hydroperoxides (Young and Woodside, 2001).

\[
\text{ROOH} + 2\text{GSH} \rightarrow \text{GSSG} + \text{H}_2\text{O} + \text{ROH}
\]

Other peroxides, including lipid hydroperoxides, can also act as substrates for these enzymes, which might therefore play a role in repairing damage resulting from lipid peroxidation. GPx require selenium at the active site and deficiency might occur in the presence of severe selenium deficiency (Nakane et al., 1998). Several GPx enzymes are encoded by discrete genes (Brigelius, 1999). The plasma form of GPx is believed to be synthesised mainly in the kidney (Roxborough et al., 1999). Within cells, the highest concentrations are found in liver although GPx is widely distributed in almost all tissues. The predominant subcellular distribution is in the cytosol and mitochondrion, suggesting that GPx is the main scavenger of H₂O₂ in these subcellular compartments. The activity of the enzyme is dependent on the constant availability of GSH (Holben and Smith, 1999). The ratio of GSH to oxidised glutathione (GSSG) is usually kept very high as a result of the activity of the enzyme glutathione reductase:

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+
\]

The NADPH required by this enzyme to replenish the supply of GSH is provided by the pentose phosphate pathway. Any competing pathway that utilises
NADPH (such as the aldose reductase pathway) might lead to a deficiency of reduced glutathione and hence impair the action of GPx. Glutathione reductase (GR) catalyses the conversion of GSSG to GSH and is a flavine nucleotide dependent, has a similar tissue distribution to GPx (Young and Woodside, 2001).

**Aqueous phase chain breaking antioxidants, including**

- Vitamin C (ascorbic acid), neutralize ROS in aqueous phase before lipid peroxidation is initiated (Jialal et al., 1990). In the cell, it is maintained in its reduced form by reaction with glutathione (Meister, 1994).
- Uric acid is present in plasma in high concentration, provides protection against certain oxidizing agents like ozone (Cross et al., 1992).
- Albumin bound bilirubin protect the neonate from oxidative damage (Gopinathan et al., 1994).
- Protein bound thiol groups, the sulphhydryl groups present on plasma proteins, albumin is the predominate plasma protein which functions as antioxidant (Halliwell, 1988).
- GSH, is a cysteine containing peptide. It is synthesized in cell from its constituent amino acids. It has direct antioxidant property, acts as an essential cofactor for GPx (Sastre et al., 1996), participates in leukotrienes synthesis and regenerates the major aqueous and lipid phase antioxidants (Atalay and Laaksonen, 2002).
- Thioredoxin, might function as a key intracellular antioxidant (Arrigo, 1999).
- Alpha-lipoic acid, exerts beneficial effect in aqueous environment, it is able to regenerate other antioxidants like vitamin C, vitamin E and GSH (Heller et al., 2001).

**The transition metal binding proteins:**

They include ferritin, transferrin, lactoferrin and ceruloplasmin which act as antioxidant defense system by sequestering iron and copper so that they are not available to drive the formation of hydroxyl radicals (Young and Woodside, 2001).

**Non-polar chain breaking antioxidants**

They are small molecules that can receive or donate an electron from or to radical with the formation of stable byproducts (Halliwell, 1995). It is divided into:
Vitamin E (alpha tocopherol), scavenges radicals in membranes and lipoprotein particles and crucial in preventing lipid peroxidation (Herrera and Barbas, 2001).

Carotenoids, most important is β-carotene, a group of lipid soluble antioxidants, as a precursor of vitamin A (retinol) which has antioxidant role (Cooper et al., 1999).

Ubiquinol-10, a reduced form of coenzyme Q-10 prevents lipids peroxidation (Shi et al., 1999). It is an endogenously synthesized compound and in higher concentration, it scavenges ROS and improves endothelial dysfunction (Johansen et al., 2005).

Flavonoids, large group of polyphenolic antioxidants found in many fruits and vegetables (Rice-Evans et al., 1996).

Concept of Oxidative stress (SOX)

The relation between free radicals and disease can be explained by the concept of ‘SOX’ elaborated by Sies (1986). In a normal healthy human body, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, over nutrition, this delicately maintained balance is shifted in favor of prooxidants resulting in ‘SOX’. It has been implicated in the etiology of several (>100) of human diseases. SOX has been generating much recent interest primarily because of its accepted role as a major contributor to etiology of both normal senescence and severe pathologies with serious public health implications. SOX is described as impairment of equilibrium between prooxidant and antioxidant systems (Rifat et al., 2009).

In physiological conditions there is equilibrium between oxidants that are generated during normal aerobic metabolism and antioxidant systems of their detoxification. Whenever additional aerobic oxidants are generated, prooxidant systems, lipids, carbohydrates, proteins and nucleic acids undergo oxidative damage and the equilibrium is broken. In the event of overwhelming radicals, the available antioxidant defense falls short and unscavenged radicals remain free, which eventually oxidises important cell components leading to SOX. Lipids, proteins and nucleic acids are the three potential molecular targets of SOX. To a lesser extent, carbohydrates are also the targets of ROS. Nucleic acids appear less susceptible than
lipids to free radical attack in that there seems less possibility of rapidly progressing, destructive chain reactions being initiated. The various free radicals and their role in mediating SOX are presented in the Table 4.

**Table 4: Molecules mediating oxidative stress (SOX)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Metabolic role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>•O–O•</td>
<td>Catalyses Haber-Weis reaction by recycling Fe^{2+} and Cu^{2+} ions, formation of hydrogen peroxide or peroxynitrite</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>HO–OH</td>
<td>Formation of hydroxyl radical, enzyme inactivation, oxidation of biomolecules</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>•OH</td>
<td>Hydrogen abstraction, production of free radicals and lipid peroxides, oxidation of thiols</td>
</tr>
<tr>
<td>Ozone</td>
<td>•O–O’=O</td>
<td>Oxidation of all kinds of biomolecules, especially those containing double bonds, formation of ozonides and cytotoxic aldehydes</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>O=O</td>
<td>Reaction with double bonds, formation of peroxides, decomposition of amino acids and nucleotides</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>•N=O</td>
<td>Formation of peroxynitrite, reaction with other radicals</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>O=N–O–O’</td>
<td>Formation of hydroxyl radical, oxidation of thiols and aromatic groups, conversion of Xanthine dehydrogenase to Xanthine oxidase, oxidation of biomolecules</td>
</tr>
<tr>
<td>Hypochlorite</td>
<td>ClO⁻</td>
<td>Oxidation of amino and sulphur containing groups, formation of chlorine</td>
</tr>
<tr>
<td>Radical</td>
<td>R•</td>
<td>Hydrogen abstraction, formation of peroxyl and other radicals, decomposition of lipids and other biomolecules</td>
</tr>
<tr>
<td>Peroxyl radical</td>
<td>R–O–O•</td>
<td>Hydrogen abstraction, formation of radicals, decomposition of lipids and other biomolecules</td>
</tr>
<tr>
<td>Hydroperoxide</td>
<td>R–O–OH</td>
<td>Oxidation of biomolecules, disruption of biological membranes</td>
</tr>
<tr>
<td>Copper and iron ions</td>
<td>Cu^{2+}, Fe^{3+}</td>
<td>Formation of hydroxyl radical by Fenton and Haber-Weis reactions</td>
</tr>
</tbody>
</table>
Association of nucleic acids with nuclear proteins reduces its accessibility to free radicals and offers additional protection (Moulakakis et al., 2008). Among these macro molecules, lipids are more susceptible to oxidative damage. It is well known that proteins are susceptible to damage by ROS and oxidative modification of proteins may lead to the structural alternation and functional inactivation of many enzyme proteins.

**Oxidative damage to lipids and proteins**

At high concentrations, ROS can be important mediators of damage to cell structures, lipids and proteins (Valko et al., 2006). It is known that metal-induced generation of ROS results in an attack on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al., 1995). Once formed, peroxyl radicals (ROO•) can be rearranged via a cyclisation reaction to endoperoxides (precursors of MDA) with the MDA (Fedtke et al., 1990; Fink et al., 1997; Mao et al., 1999; Marnett, 1999; Wang et al., 1996).

MDA is an end product of lipid peroxidation (LPO), which is a process where reactive oxygen species degrade polyunsaturated lipids. This compound is reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and for advanced glycation end products (Farmer and Davoine, 2007). The production of this aldehyde is used as a biomarker to measure the level of SOX in an organism (Moore and Roberts, 1998). The major aldehyde product of lipid peroxidation other than malondialdehyde is 4-hydroxy-2-nonenal (HNE). MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. Hydroxynonenal is weakly mutagenic but appears to be the major toxic product of lipid peroxidation.

Mechanisms involved in the oxidation of proteins by ROS were elucidated by studies in which amino acids, simple peptides and proteins were exposed to ionizing radiations under conditions where hydroxyl radicals or a mixture of hydroxyl/superoxide radicals are formed (Stadtman, 2004). The side chains of all amino acid residues of proteins, in particular cysteine and methionine residues of proteins are susceptible to oxidation by the action of ROS/RNS (Stadtman, 2004).
Oxidation of cysteine residues may lead to the reversible formation of mixed disulphides between protein thiol groups (−SH) and low molecular weight thiols, in particular GSH (S-glutathiolation). The concentration of carbonyl groups, generated by many different mechanisms is a good measure of ROS-mediated protein oxidation. A number of highly sensitive methods have been developed for the assay of protein carbonyl groups (Dalle et al., 2003; Dalle et al., 2005).

Advanced glycation end products (AGEs) is a class of complex products. They are the results of a reaction between carbohydrates and free amino group of proteins. The intermediate products are known, variously, as Amadori, Schiff Base and Maillard products, named after the researchers who first described (Dalle et al., 2005). Most of the AGEs are very unstable, reactive compounds and the end products are difficult to be completely analysed.

Myocardial ischemia (MI) and oxidative stress (SOX): Potential mechanisms

ROS contributes to the complex pathophysiology of MI. The cellular mechanisms behind ischemic injuries involve the interaction of a number of cell types, including endothelial cells, circulating blood cells (e.g., leucocytes, platelets) and cardiomyocytes (Lefer and Granger, 2000; Ceconi et al., 2000). In general, ROS mediated injury is considered to be caused by an initial burst of ROS at onset; and thereafter as the result of ROS released during inflammation. ROS has the potential to injure vascular cells and cardiomyocytes directly and can initiate a series of local chemical reactions and genetic alterations that ultimately result in an amplification of the initial ROS-mediated injury to cardiomyocytes.

Acting together, SOX and Ca^{2+} overload are proposed as two main complexly interrelated hypotheses of injury (Dhalla et al., 2000; Storti et al., 2004). When molecular oxygen is reintroduced into the ischemic myocardium, the normal balance between prooxidant and an antioxidant factor is changed. Thus the preceding depletion of ATP and further breakdown to purine catabolites like hypoxanthine (HX) and xanthine (X) and the parallel conversion of xanthine dehydrogenase to a xanthine oxidase (XO) may set the scene for a major release of superoxide.

\[
\begin{align*}
\text{ATP} & \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{Adenosine} \rightarrow \text{HX} \rightarrow \text{X} \rightarrow \text{O}_2 \rightarrow \text{Uric acid}
\end{align*}
\]
Introduction and Review of Literature

In human myocardium such a mechanism is more likely in endothelial cells than in cardiomyocytes due to the low level of xanthine dehydrogenase in the latter cell type (Opie, 1998). There is reason to believe, however, that reperfusion induced early ROS release is related to the mitochondrial respiratory chain and to an altered state of parallel antioxidant proteins in both cardiomyocytes and endothelial cells. Ischemia also leads to decrease in Na\(^+\)/K\(^+\)-ATPase activity and a rise in intracellular Na\(^+\) and Ca\(^{2+}\).

High intracellular Ca\(^{2+}\) results in ROS production due to the disruption of the mitochondrial proton gradient (Sorg, 2004; Droge, 2002). Production of O\(_2\) and all other reactive oxygen species require oxygen, which is a substance in short supply during ischemia. However, total cell anoxia is encountered infrequently in a clinical situation. Thus ROS may still be created during a prolonged period of ischemia. Complicating the issue further, ROS may induce preconditioning with an upregulation of the antioxidant defence (Bolli, 1996).

Experimental versus clinical studies of oxidative stress (SOX)

A wealth of documentation from experimental studies has shown that SOX may be a major injurious factor when oxygen is reintroduced to ischemic tissue (Cerbai et al., 1991; Ambrosio et al., 1991). Accordingly, both endogenous and exogenous antioxidants have been able to ameliorate tissue injury in experimental heart models. Some potent antioxidants used in animal experiments include: SOD or SOD mimetics; the glutathione donor N-acetyl cysteine (NAC); the iron chelator desferrioxamine; vitamins and drugs with secondary properties like the angiotensin converting enzyme inhibitor captopril and the magnetic resonance imaging (MRI) contrast agent manganese-dipyridoxyl diphasphate (Ambrosio and Flaherty, 1992; Pucheu et al., 1996; Spencer et al., 1998; Karlsson et al., 2001).

Clinical studies, however, have shown less clear cut evidence supporting the hypothesis of ROS mediated myocardial injury in situations with acute ischemia-episodes. There is overwhelming evidence implicating SOX in the long term development of IHD, but the implications are not evident. Recent primary prevention trials have investigated the role of antioxidants, mainly vitamin E or C alone or in combination, on patients at risk of CVD but the results are conflicting (Salonen et al.,
2003; Yusuf et al., 2000). In clinical studies the focus has generally been on ROS related parameters and most often indices of myocardial injury have been lacking (Buffon et al., 2000; Iuliano et al., 2001; Clermont et al., 2002). Complicating the issue further, recent research have indicated new roles of ROS as signal molecules involved in protective cellular processes and sheds doubt about the concept of SOX per se and of the value of antioxidant therapy (Marczin et al., 2003; Das and Maulik, 2004).

**Myocardial ischemia (MI) and Experimental models**

Experimental models aimed at studying MI in the integrated organism are important in human medicine given the limited possibilities for well controlled human studies. Whether the investigator wants to be close to the situation in human medicine or studies the influence of extra cardiac factors in IHD, the model of choice will be an *in vivo* model. This also includes development of new clinical methods, instruments and pharmacological agents. Most *in vivo* experimental models of MI have been established in the dog, pig, rabbit, rat or mouse. Although other species have also been used, the accumulated knowledge of the most often used laboratory animals in models of MI cannot be underscored (Kirsti, 2006).

**Ex vivo and In vitro models**

**Isolated perfused hearts**

With a steady increase over the past 20–30 years, isolated perfused hearts are used in the study of MI. The isolated perfused rat heart is used for biochemical tissue analyses, various functional measurements and evaluation of infarction size in parallel with time-limited use of pharmacological probes. With the use of mice hearts and development of transgenic animals, the use of *ex vivo* perfusion techniques will remain high. The leptin receptor deficient db/db mouse is a well described animal model for age dependent diabetic cardiomyopathy (Aasum et al., 2003).

Using mouse isolated perfused heart, the ischemia tolerance in male hearts from a well described natural genetic (db/db mouse) model of age dependent diabetic cardiomyopathy was studied. In hearts from male db/db mice reduced ischemic tolerance developed in parallel with metabolic changes (increase in the fatty acid oxidation and decrease in the glucose oxidation) (Kirsti, 2006).
Introduction and Review of Literature

Cardiomyocytes

Cardiomyocytes can be isolated acutely from hearts of all species and maintained in suspension for hours. Short time culture is also possible. Manipulation of the incubation buffer, anoxia or hypoxia, or pelleting and sealing under paraffin can be used to simulate ischemia. In this model, the importance of ROS related loss of mitochondrial membrane potential at reperfusion was demonstrated (Juhaszova et al, 2004). Cell culture techniques have considerably rationalized cell studies; cultures most often used are either based on cells isolated from neonatal or embryonic hearts of rats and mice (Claycomb et al., 1998; Okada et al., 2005) or obtained commercially as immortalised cell lines. Neonatal mice heart cell cultures can be harvested from genetically engineered mice hearts; transfection techniques as well as silencing RNA technique can be used in cell cultures. HL-1 cells are immortalised cells derived from an atrial tumour (myoma) in female mice. Compared to the use of primary isolation of ventricular myocytes, the advantages of having a good immortalised cell culture model for use in ischemia studies involve the following (Kirsti, 2006):

(1) No animal use and care.
(2) No lengthy preparation time before any experiments can take place.
(3) No variable and limited yield for high throughput approaches and
(4) No problems with heterogeneous cell population.

Myotubes or human myoblasts (girardi cells) have also been used to study ischemic preconditioning. Cells can be obtained from American Type Culture Collection (ATCC) or European Collection of Cell Cultures.

In silico models

There is limited tradition for in silico models in ischemic heart research. One exception is the electrophysiological modeling of the consequences of changes in ion balance with ischemia. Important input in these models includes potassium loss to the extracellular space, intracellular proton accumulation and opening of ATP dependent potassium channels, cellular electrical uncoupling and delayed conduction, diastolic calcium overload and increase in cellular sodium (Nygren et al., 2006). A paper by Keener (2003) describes fibrillation of the heart as a consequence of spatial heterogeneity during regional ischemia. One example related to myocardial ischemia
is the modelling of human cardiac mitochondrial metabolic network (Thiele et al., 2005). In this study, four different conditions were investigated in the normal condition and ischemia (25% reduction in oxygen supply), diabetes and diet restriction (low fat, high glucose).

**In vivo models**

*In vivo* models are usually divided into chronic or acute conscious or anaesthetised state. The majority of studies are acute ischemia in the anaesthetised animal. This requires understanding of the impact of the anaesthetic agent in use and inclusion of sham operated animals and timed controls. In the clinical situation spontaneous or intentional ischemia is regional (coronary occlusion, angioplasty) or global (lethal arrhythmias or coronary bypass surgery). Correspondingly, experimental models are aimed at simulating either global ischemia or regional ischemia models (Kirsti, 2006).

**Dog models**

Historically, experiments with MI in the anaesthetised dog have led to basic understanding of heart function and overall hemodynamic changes during regional ischemia. Techniques for measurements of oxygen consumption and substrate metabolism under ischemia combined with estimates of heart work were originally established in the in situ canine heart. Regional ischemia and infarct models were developed for testing potential cardioprotective compounds. An example demonstrating the use of established knowledge about infarction and tissue lipid metabolism in the dog heart combined with newly raised questions is the use of specific cytochrome P-450 (CYP) antagonists for cardioprotection (Nithipatikom et al., 2006). Nithipatikom et al., (2006) used regional ischemia in anaesthetized dogs to investigate the role of cytochrome P-450 (CYP-50) hydroxylases and 20-hydroxyeicosatetraenoic acids (HETE) in an infarct model.

A second model is a chronic model with a surgically implanted ameroid constrictor placed around the left coronary artery. This results in gradual narrowing of the artery lumen and corresponding gradual reduction in ejection fraction and other contractile parameters of the affected myocardium over a few weeks. The dog heart has a great potential for coronary collateral vessel growth and therefore this model is
convenient for studying the regulation of clinically important physiological adaptive angiogenesis. Thirdly, Lyseggen et al., (2005) used an open chest dog heart model to document echocardiographic indices of potential use during evaluation of reperfused myocardium. The study aim was to find means to evaluate postischemic viability and therefore whether reperfusion was successful.

Porcine models

Mainly there are two categories of models of MI in the pig heart. The first one is related to clinical cardiac surgery, advanced instrumentation and a need for mimicking the situation in human surgery. The second one includes models of acute or chronic regional ischemia like models of acute ischemic preconditioning or chronic hibernating myocardium. Acute ischemic preconditioning is the cardioprotection induced by short lasting ischemic episodes before more severe ischemic injury, for example 5 minutes ischemia followed by 5 minutes reperfusion before ischemic injury.

Recent *in vivo* studies in the dog heart have put the focus at the early phase of reperfusion (Tsang et al., 2005; Zhao et al., 2003). Transient and repetitive ischemia of 30 seconds duration applied during early reperfusion (postconditioning) has been reported to result in a significant reduction of reperfusion injury both *in vivo* and *in vitro*. Postconditioning can be applied clinically in conjunction with therapeutic reperfusion in contrast to clinical use of ischemic preconditioning which would require treatment before the disease is evident.

Rabbit models

*In vivo* models of MI in the rabbit can be acute models as well as chronic models. The uses of the rabbit heart for *in vivo* studies of regional MI, ischemic preconditioning, postconditioning and pharmacological cardio protection were reported by many workers (Tsuchida et al., 1994; Krenz et al., 2001; Philipp et al., 2006). One advantage of *in vivo* rabbit models is the possibility to transfer results from the *in vitro* isolated perfused heart into the *in vivo* setting without change of animal species. This advantage is also found in rat and mice models. Another advantage is that through special feeding regimes atherosclerotic conditions can be induced in the rabbit.
Mice models

Several experimental models developed for rats have now been adjusted for use in mice. This involves miniaturisation of equipment and, in most cases, optical aid to identify structures. The anatomical location of the left coronary artery is constant between individuals in mice (as well as in rats). Established technique for intubation and artificial ventilation makes chronic experiments with regional ischemia possible in mice because the animal can recover after surgery (Kirsti, 2006).

Rat models

The chronic in vivo rat model of regional ischemia and infarction has been used for more than three decades. When used without reperfusion, this model is mostly suited for postinfarction remodelling and failure and for the investigation of scar tissue and inflammation related to tissue repair (Qvigstad et al., 2005). For the study of infarct size limitation, reperfusion is obligate because the rat heart (like the rabbit and mice heart) has no collaterals; occlusion of blood flow to an area eventually leads to loss of myocardial cells in that area. With standardized reperfusion and quantification of infarct size, cell death delay protocols for cardioprotection are easy to establish in vivo in a rat model (Ytrehus et al., 1994).

Possibilities for hemodynamic measurements are limited in the rat heart compared to the complex instrumentation that is possible in larger animals and also during human heart surgery. However, a major improvement has come with the newest noninvasive imaging modalities, namely echo Doppler technique, nuclear magnetic resonance (NMR) imaging and spectroscopy and photon emission tomography (PET), which make possible the continuous in vivo monitoring of metabolic activity and also allows the study of the distribution of specific intra and/or extracellular tracers (Kirsti, 2006).

Isoproterenol induced myocardial ischemia

Catecholamines at low concentrations are considered to be beneficial in regulating heart function by exerting a positive ionotropic effect. Catecholamines administration at high doses or excess release of it from the endogenous stores may deplete the energy reserve of cardiomyocytes and thus may result in biochemical and structural changes which are responsible for the development of irreversible damage.
Isoproterenol (L-β-(3, 4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride), a sympathomimetic β-adrenergic receptor (β-AR) agonist, causes severe stress to the myocardium resulting in an infarct-like necrosis of heart muscle. The rat model isoproterenol (ISO) induced myocardial necrosis serves as a well-accepted standardised model to evaluate serial cardiac functions and to study the efficacy of various natural and synthetic cardioprotective agents (Rathore et al., 1998). ISO induced MI is widely accepted model for several reasons. The model is characterised by an extraordinary technical simplicity, an excellent reproducibility as well as an acceptable low mortality (Grimm et al., 1998). MI induced by ISO has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human MI (Nirmala and Puvanakrishnan, 1996). ISO-induced necrosis is maximal in the subendocardial region of the left ventricle and in the interventricular septum. Continuous infusion of ISO in rats elicits typical cardiac gene expression similar to that observed in cardiac hypertrophy caused by pressure overload (Boluyt et al., 1995).

**Mechanism of ISO-induced myocardial ischemia**

Several mechanisms for the cardiotoxic effects of high levels of ISO have been suggested. These mechanisms include:

- Functional hypoxia and ischemia.
- Coronary insufficiency.
- Alterations in lipid metabolism.
- Decreased level of high energy phosphate stores.
- Intracellular Ca²⁺ overload.
- Changes in electrolyte contents.
- Oxidative stress (SOX).

Although these changes represent individual pathological states, they are known to affect each other and thus are interpreted as complex entities. SOX is more probably, one of the main mechanisms through which catecholamines exert their toxic effects. Spontaneous oxidation of catecholamines results in the formation of catecholamine-o-quinones, which generate aminochromes through cyclisation.
Adrenochromes (which results from the cyclisation of epinephrine-o-quinone) can be oxidised to several other compounds such as adrenolutin, 5,6-dihydroxy-1-methylindol (DHMI) or adrenochrome adrenolutin dimmer. All these redox reactions generate free radicals. Consequently, catecholamine-o-quinones, aminochromes and the radical species resulting from the oxidation of catecholamines are thought to be involved in catecholamine related toxicity (Dhalla et al., 1992). The aminochrome undergo further oxidation similarly to that of adrenochrome which isomerizes to adrenolutin this oxidative reactions produce free radicals (Rupp et al., 1994). The oxidised products have the ability to interact with sulphydryl groups of various proteins and also lead to production of superoxide anions and subsequently H$_2$O$_2$. This results in changes in microsomal permeability, mitochondrial Ca$^{2+}$ uptake, decrease in ATP production and the formation of highly reactive hydroxyl radicals which causes protein, lipid damage (Bindoli et al., 1992, Dhalla et al., 2010).

ISO produces a number of biochemical and electrophysiological alterations which precede the histological changes in the heart. The primary disturbances of ISO induced MI has been reported to enhance adenyl cyclase activity resulting in increased cAMP formation, which in turn lead to the higher lipid accumulation in the myocardium. Several early events, such as ultra structural changes, histological, biochemical, electrolyte and membrane changes, have been shown to occur within 48 hr after the injection of isoproterenol. Glycogen depletion and fat deposition have been reported. Histological changes induced by excessive amounts of isoproterenol include degeneration and necrosis of myocardial fibres, accumulation of inflammatory cells, interstitial edema, lipid droplets and endocardial hemorrhage (Aman et al., 2011).

Biochemical alterations in ISO induced ischemia represent a complex pattern of changes in cardiac marker enzymes, lipid profile, lipid metabolising enzymes, enzymatic and nonenzymatic antioxidants levels, glycoprotein levels, decrease in ATP store and changes in electrolyte levels in the blood as well as in the myocardial tissue. Changes including those in sarcolemma, sarcoplasmic reticulum and mitochondria, are mainly mediated by SOX, which is known to result in alterations of enzyme activity and transport systems and cause disturbances in cellular homeostasis (Aman et al., 2011). Lipolysis is also one of the important determinants of ISO.
induced MI. Study also provides evidence that chronic β-AR stimulation markedly shows iNOS upregulation, C-reactive protein (CRP) release and nitrative stress and that iNOS-mediated nitrative stress functions as a main interface linking chronic β-AR activation and myocardial cell apoptosis (Hu et al., 2006).

Pharmacologic treatment options

A wide array of pharmacologic agents is available for the treatment of IHD. When selecting the most appropriate pharmacologic treatment for an individual patient, specific consideration should be given to agents that have been proven to improve prognosis. An in-depth discussion of these agents is beyond the scope of this thesis, rather, a brief overview of agents used to treat IHD is provided.

Beta blockers (BBs)

Blockade of β-ARs causes cardiac slowing and decreased myocardial contractility and may lower arterial pressure. These effects serve to reduce the myocardium's demand for oxygen and thus flow, especially during exercise. Patients on BBs thus require a smaller portion of their coronary flow reserve to do a given amount of physical activity and are, thus, less likely to have myocardial ischemia when coronary flow reserve is limited. Thus, after beta blockade, ischemia will occur at a higher degree of stenosis than before blockade. Not only can BBs provide symptomatic relief from angina, several trials have demonstrated that these agents can also improve survival in patients with recent or prior MI (Gibbons et al., 2003). BBs should be considered as initial therapy for chronic stable angina, secondary prevention post MI and for the reduction of mortality and morbidity in hypertensive patients. Concurrent asthma or bronchospasm and atrioventricular conduction abnormalities are relative contraindications to beta blocker therapy.

Calcium channel blockers (CCBs)

Long acting or slow release calcium channel antagonists are able to relieve the symptoms of chronic stable angina. The nondihydropyridine calcium channel blockers such as verapamil and diltiazem, reduce heart rate and should be administered with caution in patients receiving concurrent beta blocker therapy or with evidence of sinus node or LV dysfunction or AV block (Marc et al., 2004). CCBs may exert antianginal effects by decreasing heart rate at exercise and rest. These agents work by lowering
arterial pressure at rest and with exercise, thereby decreasing oxygen demand. Finally, CCBs relax vascular smooth muscle in the coronaries thereby minimising the dynamic component of angina (Marc et al., 2004).

Nitroglycerin

This agent is efficacious in relieving angina by decreasing myocardial oxygen requirements (reduced preload via systemic venodilation) and by improving myocardial perfusion (by relaxing the smooth muscle in diseased and stenotic coronary arteries). When combined with beta blockers or calcium channel blockers, nitrates can improve the antianginal efficacy of these agents (Gibbons et al., 2003). Diseased coronaries produce less NO than normal coronaries. NO is the key naturally occurring epicardial coronary vasodilator. Nitroglycerin and related long acting nitrates are NO donors and cause relaxation of vascular smooth muscle.

Antiplatelet therapy

Aspirin, the most common antiplatelet therapy, has been shown to decrease the risk of nonfatal MI in chronic stable angina patients by 33% and to reduce the risk of serious vascular events by about 25% (ATC, 2002). The antithrombotic effect of aspirin is induced by the inhibition of cyclooxygenase and the subsequent production of thromboxane A2 and prostacyclin (Bales, 2004). Patients intolerant of aspirin may be treated with clopidogrel, an antiplatelet medication that prevents adenosine diphosphate mediated activation of platelets. Clopidogrel is associated with a reduction in the combined risk of MI, vascular death, or stroke in high risk patients with established vascular disease (CAPRIE, 1996). There is little evidence that either aspirin or clopidogrel have any antianginal effect (Marc et al., 2004).

Lipid lowering therapy

The importance of an aggressive approach to the control of lipids in patients with IHD is paramount. The use of the 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors, or statins, has been associated with a decrease in mortality of up to 30% and a reduction in major coronary events in patients with known IHD of up to 35% (Sacks et al., 1996). These impressive results included some patients with baseline levels of LDL cholesterol levels under 100 mg/dL (HPTC, 2002). Reducing LDL may limit progression or even induce regression of IHD, thereby having an
Introduction and Review of Literature

indirect anti-ischemic effect. It also stabilizes plaques and thereby reduces the risk of acute ischemic syndromes (Marc et al., 2004).

Angiotensin converting enzyme inhibitors

Two recent multicenter, randomised trials—the Heart Outcomes Prevention Evaluation (HOPE) study and the European Trial on Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease (EUROPA)—demonstrated the efficacy of angiotensin converting enzyme (ACE) inhibitors in patients with IHD or those at risk of IHD (Yusuf et al., 2000; Fox, 2003). The significant effect on the incidence of cardiovascular death, MI, or stroke was reported to be independent of the blood pressure lowering effects of ACE inhibition. The results of HOPE have lead to the recommendation that ACE inhibitor therapy is appropriate for all patients with IHD and for asymptomatic patients with IHD or LV dysfunction. Some controversy exists regarding the true nature of the vascular protective effects observed with ACE inhibitor therapy in HOPE. It is possible that these cardioprotective benefits may have been due to an antihypertensive effect that is more evident when the effects of ramipril on blood pressure are examined over 24 hours rather than at a single endpoint (Marc et al., 2004).

Novel agents

Novel drug therapies for the treatment of CAD decrease anginal symptoms via optimization of myocardial energy metabolism. These therapies include carnitine derivatives, antioxidants and fatty acid oxidation inhibitors (Stanley, 2002). Perhaps the most clinically exciting of these novel agents are the fatty acid oxidation inhibitors, trimetazidine (available outside the United States) and ranolazine (currently under US Food and Drug Administration review). These agents have demonstrated an improvement in symptoms when used as monotherapy or when combined with traditional pharmacologic agents (Marc et al., 2004).

Nonpharmacologic therapies

Lifestyle modifications are important in the management of patients with IHD. Such modifications include maintenance of ideal body weight, diet, exercise and smoking cessation. In general, lifestyle modifications should be instituted prior to or
along with pharmacotherapy and should serve to complement pharmacotherapy in the control of blood pressure and dyslipidemia (American Heart Association, 2004)

Alternate therapies

Some patients with IHD require surgical intervention in addition to lifestyle modification and pharmacotherapy. In an effort to improve the prognosis of patients with IHD, the incidence of revascularisation procedures has grown dramatically over the past two decades (American Heart Association, 2004). Percutaneous coronary intervention and coronary artery bypass graft surgery are the primary treatment options for those IHD patients with high risk features or who are refractory to maximal medical management. However, many patients with IHD are not good candidates for conventional revascularisation and their management remains a clinical challenge. This is especially true of diabetics in whom IHD is often both severe and diffuse. Alternative therapies for chronic stable angina in these difficult to treat patients include surgical laser transmyocardial revascularisation (TMR), enhanced external counter pulsation (EECP) and spinal cord stimulation (Gibbons et al., 2003; Almeda et al., 2003)

The mechanisms for improvement in symptoms in patients with chronic stable angina associated with surgical TMR are currently unclear and may include increased myocardial perfusion, denervation of the myocardium, or stimulation of angiogenesis. EECP is a nonpharmacologic technique that has been found to be associated with a decrease in angina frequency and improved time to exercise induced ischemia. Spinal cord stimulation has been used since the late 1980s as a means of providing analgesia in patients with chronic angina refractory to medical, catheter based, or surgical treatment (Marc et al., 2004).

Stabilisation of plaque

It is well established that luminal narrowing by an atherosclerotic plaque contributes to the clinical manifestations of occlusive vascular disease (Shah, 2002). However, the development of an arterial thrombus superimposed on an underlying disrupted plaque is responsible for the most acute and potentially lethal manifestations of CAD. The majority of coronary thrombi occur at sites where the fibrous cap of an atherosclerotic plaque has fissured eroded, or ruptured. Although the exact
mechanisms responsible for plaque rupture are not fully defined, several features present prior to rupture have been identified. Studies have demonstrated that risk factor modification leads to a decrease in the formation of new lesions, less lesion progression and in some cases, actual regression of disease (Shah, 2002; Shah, 2003).

The magnitude of clinical event reduction observed is much greater than what can be accounted for with small changes in the severity of stenosis. Therefore, it has been postulated that risk factor modification may not change plaque mass and stenosis severity, but may change the composition of the plaque thereby reducing the propensity for plaque rupture. This "plaque stabilization" brought on by lipid modification may be due to changes in the composition of plaque and therefore a reduction in the frequency of acute vasoocclusive events. Some scientists have postulated that the depletion of lipids and decreased inflammation from atherosclerotic plaques may help to reduce the risk of plaque rupture and subsequent thrombosis via plaque stabilisation with resultant clinical benefits (Marc et al., 2004).

Synthetic drugs are expensive and a large population cannot afford these drugs. With the onset of the synthetic era, pharmaceutical industries are producing a lot of synthetic drugs that help to alleviate the ischemic diseases. With the passage of time many problems associated with frequent use of synthetic drugs become prominent like severe side effects and resistance of microbes against these drugs. In recent times research on medicinal plants has been intensified all over the world (Ahmad and Husain, 2008).

**Medicinal plants: biosynthetic laboratories for antiischemic drugs**

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Lucy and Edgar, 1999). Medicinal plants have been found as important contributors to the pharmaceutical and food industries. Natural products from plants traditionally have provided the pharmaceutical industry with one of its important sources of lead compounds in search of new drugs and medicines. The search for new
pharmacologically active agents from natural resources such as plants, animals and microbes led to discovery of many clinically useful drugs (Ahmad and Husain, 2008).

Over the past two decades, researchers have also turned to many of the traditional folk medicines-invariably a “cocktail” of natural products to uncover the scientific basis of their remedial effects which improves the efficacy as to enhance modern medical practices. India being a tropical country is blessed with vast natural resources and ancient knowledge for its judicious utilisation. However, in order to make these remedies acceptable to modern medicine, there is a need to evaluate them to identify the active principles and understand the mechanism of action. The exploitation of plants folk medicine has a long and honorable history and the use of medicinal plants still vastly exceeds the use of modern synthetic medicine. The WHO estimates that 65-80% of the world’s population use traditional medicine as their primary form of health care and 80% of traditional medicines involves plant extracts (Mueen et al., 2009).

These medicines from indigenous plants would be an immense benefit especially to inhabitants of developing countries, since the cost of these drugs would be within their means. Plants are also appreciated in pharmaceutical research as the major resource for molecules and medicines and a growing body of medical literature supports the clinical efficacy of herbal treatments. The use of herbal medicines has been increasing and is proving to be of great importance in developing countries. The public interest in medicinal plants is growing exponentially during the past decade. The use of plants for medicines still vastly exceeds the use of modern synthetic drugs. Since at one time, all drugs were obtained from natural sources. It was also linked with the initial development of the science of pharmacology, which used natural products to educate physiologic process and even define them (Mueen et al., 2009; Farnsworth, 1988).

The pharmacological evaluation of natural products therefore forms an intrinsic part of pharmacognosy. Herbal medicines are currently enjoying a revival in popularity. Ayurvedic system of medicine is spreading throughout the world with increasing population movement. Herbal medicines, as effective and potent medicines
and its compounds served as templates for the development of many drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today are derived from plants or from starting molecules of plant origin (Philipson and Anderson, 1989; Mueen et al., 2009). One major criterion for the selection of a plant for studies that are based on bioprospection of plant drugs is traditional healer’s claims for its therapeutic usefulness. It is thus worth reflecting on the cultural environment in which traditional healers use plant remedies as well as the methods of plant use, in order to strengthen the research design. Ethnobotanical input is fast becoming as a chief strategy for development of drug and the list of medicinal plants in India is endless and the country is bestowed with a plethora of plant wealth. Resurgence in the use of herbal medicines worldwide has offered an excellent opportunity to look for the therapeutic leads from our ancient system of Ayurveda that could be utilised for drug development. The recent approach towards plant drug development aims primarily at the utilisation of leads available with the ancient scriptures like Ayurveda and application of modern phytochemical techniques to understand the active principles.

The plant kingdom already furnishes many important cardioprotective drugs. The cardiac glycosides, digoxin and lanatosides, oaubin and others, which have positive inotropic effect on the heart, are still the drugs of choice for IHDs. The growing awareness of harmful effects of chemotherapy pursued people to explore the time tested remedies from traditional alternative medicine (Williamson, 1998). With the advancement of research in medicine, it was concluded that plants are biosynthetic laboratories for chemical compounds, which are responsible for curative action of plants.

**Cardioprotective pharmacology of medicinal plants**

Cardiovascular medicines have evolved steadily over the past two decades with emphasis placed on prevention, early diagnosis and aggressive intervention. Herbs have been used as medical treatments since the beginning of civilisation and for cardiac diseases, like MI, congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis and arrhythmia. Constant research is necessary to elucidate the biological activities of the medicines now being used to treat CVDs (Miller, 1996). *Berberis aristata* fruit extract exhibited a positive inotropic action in isolated
cardiac tissues. Organic solvent fractionation revealed that the cardiotonic activity was concentrated in butanolic fraction. The presence of an active principle present in Berberis aristata caused a selective inotropic effect involving actin, myosin cooperative mechanism leading to isolation of cardioactive compound (Gilani et al., 1999).

Hirai et al., (1997) investigated cardiotonic effect of the rhizomes of Polygonatum sibiricum. Cardiovascular actions of the volatile oil obtained from black seeds (Nigella sativa) were studied, involving the effects on the arterial blood pressure and heart of urethane anaesthesised rats in comparison with those of its constituent thymoquinone. Mechanism of action elucidated that cardiovascular depressant effects were mediated mainly centrally involving 5-hydroxytryptamine and muscuranic mechanism (El-Tahir et al., 1993). Abdalla et al., (1993) studied the positive inotropic effects of oblongine alkaloids, an alkaloid from Leontice leontopetalum on guinea pig isolated muscle and heart. The ethanolic extracts of the roots of Cryptolepsis sanguenilonia with its main constituent cryptolepine were isolated by column chromatography and a negative chronotropic effects were displayed (Rauwald et al., 1992).

Tanghinin and its acetylated derivative acetyl tanghinin, two cardiotonic glycosides isolated from Tanghinia venenifera were evaluated and found that higher concentrations possessed inotropic effect, which was dropped rapidly with a rise in a diastolic tension (Randimbivololona and Rakotomanga, 1990). Taesotikul et al., (1998) investigated the effect of crude alkaloid fraction from the stem of Tabernanthe pandacaqui on the blood pressure and heart rate in conscious and anaesthetised rats. Their findings suggested that the hypotensive and bradycardiac response of the first phase involved cholinergic and central mechanism whereas the second phase involved mechanism mediated by central biogenic amines acetyl choline and histamine.

Desai et al., (1998) isolated 13 new derivatives of diterpenoid alkaloids and established their structures. Preliminary in vivo cardiovascular actions such as hypotension, bradycardia and ventricular arrhythmias were tested using male Sprague Dawley rats. A highly selective β1- adrenergic blockade with partial β2- agonist
activity was observed from ferulic acid. An active component of *Ligusticum wallichii* was evaluated using Wistar rats for cardiovascular activities. It was observed that ferulolinol markedly inhibited tachycardial effects induced by isoproterenol but no blocking effect on the arterial pressure responses induced by phenylephrine. The above findings suggested the ferulolinol possess β-adrenergic blocking activity (Wu et al., 1998).

Heubach and Schule, reported that the analgesic compound lappaconitine, a C19 diterpenoid alkaloid from *Aconitum sinomonatanum* is an inhibitor of voltage dependent sodium channels. They investigated the cardiac effects of lappaconitine and it’s metabolite in electrically stimulated guinea pig heart. It was observed that the compounds exerted negative inotropic action and concluded that lappaconitine is a naturally occurring compound with class-I anti-arrhythmic action (Heubach and Schule, 1998). The cardiotonic effect of the rhizome of *Polygonatum sibiricum* was investigated in the left atria of rats. Methanolic extract in a concentration 1-7 mg/mL was found to strongly inhibit cAMP phosphodiesterase. The findings suggested that the cardiotonic effect was due to stimulation of β-adrenoreceptors through stimulation of sympathetic nerves (Hirai et al., 1997).

Effect of trilinolein, a triacylglycerol isolated from *Panax pseudoginseng* was studied on SOD activity and left ventricular pressure in isolated rat heart subjected to hypoxia and normoxic perfusion. Better preservation was observed with trilinolein and myocardial protection was related to an antioxidant effect through potentiation of SOD (Chan et al., 1997). Examination of the actions at tissue level revealed the (*Ruta graveolens*) rue had positive chronotropic and positive inotropic effects. A number of mechanisms identified suggested that these plants contained cardioactive substances having direct effect on cardiovascular system (Chiu and Fung, 1997). Elucidation of cardiovascular activities of andrographolide and aqueous fraction of *Andrographis paniculata* for the first time was carried out by Zhang and Tan (1997) in anaesthetised Sprague Dawley rats.

Sympathomimetic activity of an ethanolic extract of *Scoparia dulcis* was investigated in rodent in vivo and in vitro preparation. High performance liquid chromatographic analysis of the aqueous fraction revealed that the presence of both
noradrenaline and adrenaline in the plant extract and the results indicated that both catecholamines accounted for the hypertensive and inotropic effects obtained after parenteral administration of extracts (Freire et al., 1996). Effect of the aqueous extracts of the bark of Terminalia arjuna on coronary flow in isolated perfused rabbit heart preparation was reported by Bhatia et al., (1998). It was found to increase the coronary flow supporting its clinically reported antianginal activity and its use as a cardioprotective.

Influence of the flavonoids from Crataegus species on coronary flow, heart rate and left ventricular pressure, velocity of contraction and relaxation was investigated using Lagendroff’s perfused isolated guinea pig hearts. Results suggested that, an inhibition of the 3,5-cyclic adenosine monophosphate phosphodiesterase was the underlying mechanism of cardiac action (Schussler et al., 1995). Identification of coumarins from natural source and study of therapeutic application depending on the pattern of substitution was done by Hoult and Paya (1996). Of these, Osthole from Angelica pubescens caused hypotension in vivo, inhibited platelet aggregation and smooth muscle contraction in vitro. Simple coumarins were found to be scavengers of superoxide anion radicals and aqueous alkyl peroxyl radicals and both 5,7 and 6,7-dihydroxy 4-methylcoumarin were found to reduce the derivation of ventricular fibrillation in post ischemic reperfused isolated rat hearts. There are several reports on effectiveness of medicinal plants against ISO induced MI in experiment animals. Table 5, depicts the list of natural medicine which has been proved to prevent ISO induced MI.

Antioxidants from medicinal plants

Owing to the incomplete efficiency of our endogenous defence systems and the existence of physiopathological situations (ischemia, cigarette smoking, air pollutants, UV radiation, high polyunsaturated fatty acid diet, inflammation, reperfusion injury, etc) in which ROS are produced in excess and at the wrong time and place, dietary anti-oxidants are needed for diminishing the cumulative effects of oxidative damage over the life span (Tiwari, 2001).

Well established antioxidants derived from the diet are vitamins C, E, A and carotenoids, which have been studied intensely. Besides these antioxidant vitamins,
other substances in plants might account for at least a part of the health benefits associated with the vegetable and fruit consumption. Over the past decade evidence has been accumulated that polyphenols are an important class of defence antioxidants. These compounds are widespread actually in all plants with often at high levels including flavonoids, phenolics, phenolic acids, tannins and lignans (Pietta, 2000).

**Flavonoids as natural antioxidants**

Flavonoids are formed in plants from phenylalanine, tyrosine and malonate. The basic flavonoids structure is the flavan (2- phenyl- benzopyran) nucleus, which consists of 15 carbon atom arranged in three Rings. The O-dihydroxy catechol structure in ring B, which is the obvious radical target site for all flavonoids, with a saturated 2,3 double bond. The 2,3 double bond in conjugation with an oxo-function which is responsible for electron delocalisation from the B ring. The additional presence of both 3 and 5 hydroxyl groups for maximum radical scavenging potential and strong radical absorption (Mueen et al., 2009).

ROS are implicated in many pathogenic processes including cardiovascular system. Detoxification of ROS by antioxidants affords protection against such diseases. There is a growing body of evidence suggesting that antioxidants contribute to cardioprotection. Miura et al., (1998) suggested that flavonoids which possess pyrogallol and catechol moieties in their structure show strong H2O2 generating activity via an O2 anion radical and also possess inhibitory activities in rat liver microsomal lipid peroxidation. Flavonoids which generate H2O2 can scavenge free radicals. Antioxidant activity of 24 ferulic acid related compounds together with 6 gallic acid was evaluated by using several physical systems as well as their radical scavenging activity by Kikuzaki et al., (2002). Ferulic acid was found to be most effective antioxidant among the tested phenolic acids. Kim et al., (2002) investigated the free radical scavenging activity of *Panax ginseng* using electron spin resonance spectrometer and spin trapping techniques. Hydroxyl radical and superoxide radicals generated by UV radiation were significantly scavenged by ginseng.

**Protection of flavonoids from ischemia**

The most well known protection of flavonoids from ischemic injury is conferred by their direct antioxidant activities. Nevertheless, there are other
antioxidant effects that are delivered through different mechanisms such as posttranslational modulation of enzymes and induction of genes. Although the mechanisms involved are uncertain, there is evidence that flavonoids inhibit ROS generation during heart ischemia. For instance, three weeks feeding with grape seed proanthocyanidins decreased the electron spin resonance detectable generation of free radicals during ischemia (Pataki et al., 2002). Furthermore, flavonoids have been shown to decrease ischemia induced oxidative damage in myocardium. For instance, perfusing hearts with quercetin for 30 min and more strongly oral treatment with quercetin for 1 week before ischemia reduced MDA levels in heart tissues (Ikizler et al., 2007). Similarly, 30 days feeding rats with either skin or flesh of red grapes attenuated formation of MDA in ischemic hearts (Falchi et al., 2006).

**Reactive oxygen species scavenging activities**

Flavonoids may protect heart from ischemia injury by scavenging ROS. Flavonoids are potent scavengers of reactive species such as superoxide (Chun et al., 2003; Jovanovic and Simic, 2000), peroxyl radicals (Nakao et al., 1998; Boadi et al., 2005) and peroxynitrite (Pollard et al., 2006). By scavenging such reactive species, flavonoids prevent formation of highly reactive species of oxygen and limit perpetuation of oxidative reactions. Moreover, scavenging ROS bestows additional benefits.

For instance, by scavenging superoxide radicals the bioavailability of nitric oxide (NO) increases (Shutenko et al., 1999; Freedman et al. 2001; Benito et al., 2002) and endothelial function in postischemic hearts improves. Also, peroxynitrite is a highly reactive species of oxygen involved in cardiac injury (Lalu et al., 2002; Szabo and Bahrle, 2005; Falk et al., 2007). Peroxynitrite can cause endothelium dysfunction through nitration of the nitric oxide synthase (NOS) cofactor, tetrahydrobiopterin, which in turn uncouples NOS and produces more ROS and also through nitration and inhibition of prostacyclin synthase (Munzel et al., 2008; McCarty, 2008). Thus, scavenging superoxide and peroxynitrite by flavonoids may help to prevent endothelial dysfunction.

Vitamin C and glutathione in the aqueous phase and vitamin E in the lipid phase are likely to be much more important as direct scavengers of ROS.
Nevertheless, flavonoids at nanomolar concentrations are found to protect cultured cells against reactive species such as peroxynitrite (Serraino et al., 2003). Dietary supplementations with flavonoids have been shown to inhibit LDL oxidation in both *in vivo* and *ex vivo* settings (Aviram et al., 2002; Kasaoka et al., 2002).

However, it is difficult to ascertain if these effects are from direct scavenging of ROS or from other mechanisms. The levels of flavonoids that are achievable in heart tissues are not well known, although quercetin metabolites have been found deposited in human aorta (Terao et al., 2008). Flavonoids at relatively low concentrations may become important antioxidants in microenvironments that are less accessible to vitamin C and vitamin E, such as at the interface of membranes (Bandy and Bechara, 2001).

**Inhibition of xanthine oxidase**

Inhibition of xanthine oxidase may be one of the mechanisms by which flavonoids at physiological concentrations can mitigate ischemia injury. Several flavonoids including luteolin, apigenin, quercetin, myricetin and kaempferol have been shown to inhibit xanthine oxidase (Van-Hoorn et al., 2002; Lin et al., 2002; Mo et al., 2007). Particularly in coronary vessels and interstitial cells where xanthine oxidase activity is thought to participate in ischemia injury (Ashraf and Samra, 1993), inhibiting xanthine oxidase may help to prevent formation of superoxide (O$_2^{-}$).

**Inhibition of NADPH oxidases**

NADPH oxidases are membrane associated enzymes which catalyse transfer of one electron from NADPH to O$_2$ with consequent generation of O$_2^{-}$ (Griendling et al., 2000, Cave et al., 2005). Although NADPH oxidase was originally thought to be a neutrophil enzyme, recent investigations showed expression of NADPH oxidases in cardiovascular cells, including cardiac cells, endothelial and smooth muscle cells and fibroblasts. The expression of subunits (Fukui et al., 2001) and the enzyme activity (Heymes et al., 2003) of NADPH oxidase has been shown to increase in infarcted myocardium and failing hearts and may contribute to ventricular remodeling and cardiac hypertrophy (Looi et al., 2008). NADPH oxidases likely bring benefits to ischemic myocardium by promoting myocardial angiogenesis (Chen et al., 2007).
Introduction and Review of Literature

Although not yet investigated for this mechanism in ischemia, flavonoids have shown ability to suppress enzyme activity and/or expression of NADPH oxidases in other types of stress. For instance, epigallocatechin gallate inhibited expression of NADPH oxidase subunits in neonatal rat cardiomyocytes induced by angiotensin II and in rat hearts subjected to the pressure overload (Li et al., 2006). Similarly, the dietary administration of anthocyanins, proanthocyanidins, or catechin oligomers for 6 weeks lowered cardiac NADPH oxidase expression in rats treated with high fructose diet (Al-Awwadi et al., 2005). Likewise, diminished activity of NADPH oxidase was observed in neutrophils of hemodialysis patients who consumed concentrated red grape juice for two weeks (Castilla et al., 2008). Interestingly, inhibition of the NADPH oxidase of endothelial cells has recently been proposed as a mechanism by which catechins improve vascular function (Schewe et al., 2008), which could be of benefit in protecting against ischemia injury.

Reinforcement of cellular antioxidants

Human studies have shown depletion of non-enzymatic antioxidants such as glutathione, ascorbic acid and vitamin E following myocardial ischemia (Marczin et al., 2003). Hydrophilic antioxidants, such as ascorbate and glutathione, have shown to work at the front line of defense against SOX, protecting lipophilic antioxidants such as ubiquinol and vitamin E from oxidation (Haramaki et al., 1998). Ascorbic acid also helps to regenerate vitamin E from its oxidised form (Nagaoka et al., 2007) and is in turn recycled by glutathione (May et al., 1996), although vitamin C is also needed for the recovery of glutathione from its oxidised form (Montecinos et al., 2007).

In such a network, flavonoids are proposed to act as intermediate antioxidants, protecting lipophilic antioxidants and being protected by hydrophilic antioxidants (Lotito and Fraga, 2000). Despite considerable progress in the management of MI by synthetic drugs, the search for indigenous natural antiischemic agents is still going on. Many indigenous drugs are claimed to possess cardioprotective activity.

Ethnomedicinal information revealed by the indigenous people of the tribes of Chittoor Dt, Andhra Pradesh, India on one such plant i.e., Acalypha indica, claimed for the treatment of heart related diseases. However, there is no scientific confirmation of this claim. Recently flavonoids, notably the kaempferol glycosides
have been isolated from the flowers and leaves of *Acalypha indica*. Therefore, keeping the above concept in mind, using this plant, our study was designed to assess the effect of flavonoid rich extract on myocardial ischemia in rats.

*Acalypha indica* L.

*Acalypha indica* L. is an annual erect herb found throughout various parts of India, Bangladesh, Sri Lanka, the Philippines and tropical Africa. It was formerly listed in the British Pharmacopoeia. It has numerous medicinal uses and is listed in the Pharmacopoeia of India as an expectorant to treat asthma and pneumonia. In India and Indonesia the plant is cultivated for its edible shoots and leaves, which are cooked as a vegetable. The plant has wide uses in the traditional medicines of various countries and reportedly possesses diuretic, purgative and antihelmintic properties, besides being also used for bronchitis, asthma, pneumonia, scabies and other cutaneous diseases. A drug used for prevention and reversal of atherosclerotic disease process in the Sidha system of Indian medicine, anna pavala sindhooram, contains the leaves of this plant as one of the ingredients (Kirtikar and Basu, 1999).
Botanical name: *Acalypha indica* Linn.
Family: Euphorbiaceae
Genus: *Acalypha*
Common name: Kuppichettu; Harita-manjiri; Kuppinta or Muripindi.
Species: *indica*
Parts used: Leaves, root, stalks (young shoots) and flowers.

Chemical constituents reported from this plant include acalyphamide (as acetate), aurantiamide and its acetate, succinimide acalypholactate, 2-methyl anthraquinone, tri-O-methylellagic acid, β-sitosterol and its β-D-glucoside (leaves); a cyanogenic glucoside, acalyphine, two alkaloids, viz, acalyphine which is a 3-cyanopyridone derivative and triacetonamine, an essential oil n-octacosanol, kaempferol, quebrachitol, β-sitosterol acetate and tannin (whole plant); stigmasterol (root) (Raj and Singh, 2000). Flavonoids, notably the kaempferol glycosides mauritianin, clitorin, nicotiflorin and biorobin, have been isolated from the flowers and leaves. The plant also contains tannins, β-sitosterol, acalyphamide, aurantiamide, succinimide and the pyranoquinolinone alkaloid flindersin. Recently, four kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin have been isolated from the flowers and leaves of this plant (Nahrstedt et al., 2006).

Aqueous residues of the plant have been reported to demonstrate antidiabetic activity (Manisha et al., 2011) and antibacterial activity against *Aeromonas hydrophila* and *Bacillus cereus* (Perumal et al., 1999). Petroleum ether and ethanol extracts of the whole plant demonstrated postcoital antifertility activity in female albino rats. (Hiremath et al., 1999). Ethanolic extract of the plant showed promising wound healing activity in rats (Reddy et al., 2002). Administration of ethanol leaf extract of the plant has been shown to inhibit significantly the *Viper russelli* venom induced lethality, haemorrhage, necrotising and mast cell degranulation in rats in a dose dependent manner and cardiotoxic and neurotoxic effects in isolated frog tissue (Shirwaikar et al., 2004). The aqueous and ethanolic extracts of *Acalypha indica* leaves were also investigated for its antioxidant activity in animal models of gastric ulcer (Ramachandran et al., 2008). It is evident from the literature that the medicinal properties of *A.indica* are not exploited properly. Antiischemic potential of this plant need to be explored.
### Table 5: Medicinal plants and their efficacy against myocardial ischemia.

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salvia miltiorrhiza</em></td>
<td>Improved cardiac marker enzymes, antioxidant enzymes, mitochondrial dysfunction, left ventricular function, ECG and histopathological alterations.</td>
<td>Wang et al., 2009.</td>
</tr>
<tr>
<td><em>Mangifera indica</em></td>
<td>Prevents serum cardiac marker enzymes, enzymatic and nonenzymatic antioxidants, mitochondrial alterations, oxidation with energy metabolism and restore TCA cycle enzyme activities.</td>
<td>Prabhu et al., 2006a,b.</td>
</tr>
<tr>
<td></td>
<td>Prevented heart weight, body weight, serum marker enzymes, lipid peroxidation, endogenous antioxidants, ATPases, serum and heart lipid profile, lipid metabolising enzymes and histopathological alterations</td>
<td>Upaganlawar et al., 2009.</td>
</tr>
<tr>
<td><em>Tribulus terrestris</em></td>
<td>Protected biochemical, hemodynamic and ultrastructural changes.</td>
<td>Ojha et al., 2008.</td>
</tr>
<tr>
<td><em>Curcuminis trigona</em></td>
<td>Serum marker enzymes, electrocardiographic changes (ST elevation, QRS complex, P wave, RR interval and heart rate) and histopathological changes are well protected.</td>
<td>Thippeswamy et al., 2009.</td>
</tr>
<tr>
<td><em>Premna serratifolia</em></td>
<td>Maintained changes in ECG, albumin/globulin ratio, heart tissue proteins, glycogen, nucleic acids and blood glucose.</td>
<td>Rajendran and Basha (2008).</td>
</tr>
<tr>
<td><em>Tender coconut water</em></td>
<td>Lipid profile, serum marker enzymes and histopathological changes were improved.</td>
<td>Anurag and Rajamohan (2003).</td>
</tr>
<tr>
<td><em>Picrorrhiza kuruoa</em></td>
<td>Improved serum and tissue lipid profile.</td>
<td>Kumar et al., 2001.</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td><em>Gleditsia sinensis</em></td>
<td>Prevented ST segment changes and Bcl-2 mRNA levels</td>
<td>Wu et al., 2010.</td>
</tr>
<tr>
<td><em>Embelia ribes</em></td>
<td>Heart rate, systolic blood pressure, serum marker enzymes, endogenous antioxidants, histopathological alterations were improved.</td>
<td>Bhandari et al., 2008.</td>
</tr>
<tr>
<td><em>Desmodium gangeticum</em></td>
<td>Serum and tissue marker enzymes, lipid profile, lipid metabolising enzymes, endogenous antioxidants.</td>
<td>Kurian et al., 2005.</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>Improved mean arterial blood pressure, systolic arterial pressure, diastolic arterial pressure, heart rate. Maintained cardiac marker enzymes, lipid profile and histopathological alterations.</td>
<td>Peer et al., 2007.</td>
</tr>
<tr>
<td><em>Calotropis procera</em></td>
<td>Maintained the levels of marker enzymes, lipid peroxides, glutathione content and histopathological changes.</td>
<td>Ahmed et al., 2004.</td>
</tr>
<tr>
<td><em>Curcuma longa</em></td>
<td>Lyosomal hydrolases, cathepsin B, cathepsin D, acid phosphatase were protected. Maintained myocardial marker enzymes, lipid peroxidation and antioxidants, histopathological alterations. Restored cardiac function as evident by improved contractile functions, decreased left ventricular end-diastolic pressure, restored arterial pressure and heart rate. It also show stabilisation of cytoskeleton structure which in turn attributed to Hsp27 expression, decreased the degree of the existing collagen matrix and collagen synthesis.</td>
<td>Nirmala and Renganjulu (1996).</td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td>Corrected the iron content, plasma iron binding capacity, ceruloplasmin activity and glutathione levels, lipid peroxides and antioxidant enzymes.</td>
<td>Saravanan and Prakash (2004).</td>
</tr>
<tr>
<td><em>Crataegus oxyacantha</em></td>
<td>Prevented the defective oxidative phosphorylation and diminished energy production, mitochondrial krebs’s cycle enzymes, lipid peroxidation, antioxidants status, ultrastructural changes</td>
<td>Jayalakshmi and Devaraj (2004).</td>
</tr>
</tbody>
</table>

67
### Introduction and Review of Literature

<table>
<thead>
<tr>
<th>Plant</th>
<th>Activity Description</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oxalis corniculata</em></td>
<td>Maintained cardiac injury marker enzymes, serum lipids, lipogenic enzymes, glucose-6-phosphate dehydrogenase, antioxidant enzymes, histopathological alterations.</td>
<td>Abhilash et al., 2011.</td>
</tr>
<tr>
<td><em>Crocus sativus</em></td>
<td>Modulated significantly hemodynamic parameters and significant decrease in maximum positive and negative rate of developed left ventricular pressure and an increase in left ventricular and end-diastolic pressure and antioxidant levels and histopathological alterations and ultrastructural changes.</td>
<td>Goyal et al., 2010.</td>
</tr>
<tr>
<td><em>Moringa oleifera</em></td>
<td>Demonstrated mitigating effects on hemodynamic parameters and modulated biochemical enzymes but failed to demonstrate any significant effect on reduced glutathione level. Treatment significantly prevented the rise in lipid peroxidation and histopathological alterations in myocardium.</td>
<td>Nandave et al., 2009.</td>
</tr>
<tr>
<td><em>Commiphora mukul</em></td>
<td>Preserved the structural integrity of myocardium. Reduced the leakage of LDH and maintained structural integrity of myocardium along with favorable modulation of cardiac function and improved cardiac performance.</td>
<td>Ojha et al., 2008.</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>Pretreatment restored myocardial antioxidant status and most of the altered hemodynamic parameters.</td>
<td>Mohanty et al., 2004.</td>
</tr>
<tr>
<td>Terminalia arjuna</td>
<td>Effectively maintained serum enzyme levels and ECG changes towards normalcy. Prevented the SOX and histopathological alterations.</td>
<td>Sumitra et al., 2001.</td>
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<tr>
<td>Momordica cymbalaria</td>
<td>Prevented the elevation of serum marker enzymes and the alterations in the SOX markers in rats.</td>
<td>Raju et al., 2009.</td>
</tr>
<tr>
<td>Sida rhomboidea</td>
<td>Pretreatment showed significant decrease in heart weight, plasma lipid profile, marker enzymes, lipid peroxidation, ATPases, antioxidants.</td>
<td>Thounaojam et al., 2011.</td>
</tr>
<tr>
<td>Punica granatum</td>
<td>Prevented altered ECG pattern, enzyme markers, heart rate, vascular activity, infarct size, histopathological changes, SOD, CAT.</td>
<td>Mohan et al., 2010.</td>
</tr>
<tr>
<td>Crocus sativus</td>
<td>Prevented altered MDA level and GPx and SOD activities, troponin I, along with histopathological alterations in heart tissue.</td>
<td>Joukar et al., 2010.</td>
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</tbody>
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