1. INTRODUCTION

Global status report of world health organization (2011) stated that Non-communicable diseases (NCD) are the leading global causes of death than all other causes. NCD strike hardest at the world’s low- and middle-income populations (WHO, 2011a). Among the NCD, cardiovascular diseases (CVD) are the number one cause of death globally accounting almost 30% of the total death causes. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. More than 80% of CVD deaths takes place in low- and middle-income countries and occur almost equally in men and women. Of the 17.3 million CVD deaths in 2008, 7.3 million were due to coronary heart disease and 6.2 million were due to stroke (WHO, 2011b).

Statistics released from World Health Organisation in the year 2013 has revealed that cardiovascular diseases and diabetes stands first by contributing 32% of mortality in India at the age group of 30-70 (WHO, 2013). The leading risk factors globally for mortality is raised blood pressure (responsible for 13% of deaths globally), followed by tobacco use (9%), raised blood glucose (6%), physical inactivity (6%), and overweight and obesity (5%) (WHO, 2009). Four categories of cardiovascular diseases such as ischemic heart disease, hypertension, valvular diseases, and congenital heart diseases accounts for about 85-90 % of all cardiac deaths. Among them ischemic heart disease contributes the major percentage of mortality and disability.

1.1 ISCHEMIC HEART DISEASE

Ischemic heart disease (IHD) is the generic designation for a group of closely related syndromes which result from ischemia – an imbalance between the supply and
demand of the heart for oxygenated blood. Ischemia comprises not only of insufficiency of blood supply but also reduced availability of the nutrient substrates and inadequate disposal of metabolites. Since, coronary artery narrowing or obstruction owing to atherosclerosis underlies myocardial ischemia in a vast majority of the patients, IHD is often termed as coronary artery disease (CAD) or coronary heart disease (CHD).

Depending upon the rate of development and ultimate severity of the arterial narrowing and the myocardial response, four ischemic syndromes such as angina pectoris, myocardial infarction, chronic ischemic heart disease and sudden cardiac death may result. Myocardial Infarction is overwhelmingly the most important form of IHD.

1.2 Epidemiology and Risk Factors of IHD

Suffice to say that myocardial infarction may occur at virtually any age, but the frequency rises progressively with increasing age. Five percent of myocardial infarcts occur in people under age 40 years and 45 % occur under age of 65. Blacks and white are affected often equally. Throughout life, men are at significantly greater risk of myocardial infarction than women. However, the difference is progressively declining with advancing age. Except for those having some predisposing atherogenic conditions, women are remarkably protected against myocardial infarction during reproductive life. The uses of oral contraceptives, as formulated in the past have increased the risk of myocardial infarction especially among smokers older than age 35. Newer formulations have markedly reduced estrogen content with consequent reduction of risk at all ages. Moreover, epidemiological evidence strongly suggests that hormone replacement therapy protects postmenopausal women against myocardial infarction through favourable adjustment of risk factors (Chilvers et al., 2003).
1.3 **RISK FACTORS OF IHD**

The risk factors that predispose to atherosclerosis and resultant IHD have been identified by means of number of prospective studies in well defined population groups. Remarkably the famed Framingham study and others (Neaton and Wentworth, 1992; Lanas *et al*., 2007) have established various risk factors. Among the various factors, four factors are considered as the major risk factors, namely hyperlipidemia, hypertension, cigarette smoking and diabetes.

1.3.1 **HYPERLIPIDEMIA**

Hypercholesterolemia and other abnormalities in lipid metabolism contribute a major risk factor in atherosclerosis. The evidence linking hypercholesterolemia and atherosclerosis takes many forms:

- Atherosclerotic plaques are rich in cholesterol and cholesterol esters, which are derived largely from lipoproteins in blood.
- Lesions of atherosclerosis can be induced in many experimental animals, including subhuman primates, by feeding those diets that raised the plasma cholesterol level.
- Genetic disorders causing severe hypercholesterolemia lead to premature atherosclerosis, often fatal in childhood, despite the absence of any other risk factor. Acquired diseases that cause hypercholesterolemia, such as the nephritic syndrome and hypothyroidism, also increase the risk of IHD.
- With few exceptions, populations having relatively high levels of blood cholesterol have higher mortality from IHD. Many of the studies indicate the increasing risk with increasing serum cholesterol concentrations (Hornung, 2002).
No single level of plasma cholesterol identifies those at risk. The higher the level is the higher the risk. The risk raises more steeply, once, a plateau level of approximately 200 mg/dl is exceeded. The most striking association is with elevated levels of low-density lipoprotein (LDL), the lipoprotein moiety rich in cholesterol; however, hypertriglyceridemia with increased concentrations of very low-density lipoprotein (VLDL) also appears to increase the risk. In contrast, serum levels of high-density lipoprotein (HDL) are inversely related to risk: the higher the level is the lower the risk. Thus, HDL is often called the “good cholesterol”.

High dietary intake of cholesterol and saturated fats, such as those present in egg yolk, animal fats and butter, raises the plasma cholesterol level. Conversely, a diet low in cholesterol and low in the ratio of saturated-to-polyunsaturated fats lowers plasma cholesterol levels. Paradoxically, Greenland Eskimos who have a high dietary fat consumption, have low rates of IHD. This is thought to be due to the high content of omega-3 fatty acids in their diets. Such fatty acids have a number of anti-atherogenic effects, including the lowering of plasma LDL, increasing plasma HDL and modifying production by blood and vascular cells mediators that affect platelet function and inflammation.

1.3.2 Hypertension

Although the mechanism is not entirely clear, elevated blood pressure unequivocally accelerates atherogenesis and increase the incidence of IHD (Glazer et al., 2005). Both diastolic and systolic hypertensions are deleterious. A trial study in the multiple risk factor intervention has revealed the increased death rates to the association with systolic blood pressure above 110 mm Hg and diastolic pressure greater than 70 mm
Hg. After 45 years of age, hypertension is a stronger risk factor than hypercholesterolemia. Antihypertensive therapy reduces the incidence of strokes and IHD.

1.3.3 Cigarette Smoking

Smoking is firmly established as a risk factor in diseases caused by atherosclerosis (Law and Wald, 2003). Smoking is the dominant cause of the increased incidence of IHD among women, a shoot up in the incidence of sudden death among victims of heart attacks, and a rise in the degree of aortic and coronary atherosclerosis at autopsy. Cessation of cigarette in high risk individuals is followed within a few years by a reduction in the risk of dying of IHD.

1.3.4 Diabetes

Patients with diabetes has twofold increase in the incidence of myocardial infarction than non-diabetics, an increased tendency toward cerebral thrombosis and infarction, and an 8 fold to 150 fold increased frequency of gangrene of lower extremities. There is also an increased frequency of atherosclerosis among diabetic patients compared with non-diabetic (Aguilar et al., 2004).

Other risk factors include insufficient regular physical activity, stressful lifestyle, obesity and, use of oral contraceptives, hyperuricemia, high carbohydrate intake and hyperhomocysteinemia (Mennen et al., 2002). Each of the major risk factors noted earlier contributes individually to the possible development of clinically significant atherosclerosis, but multiple factors exert a synergetic effect.
1.4 Patho-physiological links among risk factors, oxidative stress and atherothrombosis

Risk factors such as hypertension, hyperglycemia and hyperlipidemia have certain things in common, that is “oxidative stress”, through which they progress and lead to atherothrombosis.

Hypertension enhances the vascular production of superoxide independently of the renin-angiotensin system (Beswick et al., 2001). The angiotensin system activation is associated with vascular production of $\text{O}_2^\cdot$ (Berry et al., 2000). Either the first or the second mechanism, induction of oxidative stress occurs via the activation of NAD(P)H oxidase enzyme. The relation between oxidative stress and hypertension has been established in humans (Germano et al., 2004) and has been found that patients with hypertension have enhanced platelet formation mediated by AT1 receptors, as well as NAD(P)H-oxidase that plays a pivotal role in enhanced formation of oxygen-free radical production. Enhanced oxidative stress by the vessel wall reduces the vasodilatory property of endothelium inhibiting NO activity (Kaliora et al., 2006) thus, inducing progression of atherosclerotic disease.

Endothelium dependent vasodilation is reduced in patients with diabetes and that vitamin C is able to prevent it, meaning a role for oxygen-free radicals in reducing the vasodilatory property of endothelium (Timimi et al., 1998). Hyperglycaemia enhances oxidative stress and induces vascular damage via several pathways, including the formation of the advanced glycated end products (AGE) that are pro-atherogenic and pro-thrombotic substances. Furthermore glucose alters the balance between free radicals in endothelial cells and NO does not exert its vasodilatory and antioxidant effect (Cosentino et al., 1997). The mechanism by which hyperglycemia induces oxidative stress, is
NAD(P)H oxidase and COX activation (Wolin, 2000), vascular expression of NAD(P)H oxidase has been found over expressed in diabetic patients (Guzik et al., 2002).

Hypercholesterolemia is associated with enhanced oxidative stress. For example, free radicals and F2 isoprostanes have been found to be elevated in the arteries of hypercholesterolaemic animals (Ohara et al., 1995) or in the urine of patients with high serum cholesterol, respectively (Davi et al., 1997). Cholesterol has been shown to activate the metabolism of the arachidonic acid pathway (Sanguigni et al., 2002), which is associated with NAD(P)H oxidase activation (Wolin, 2000). When treating hypercholesterolaemic patients with an inhibitor of HMG-CoA-reductase reduced formation of TNF-α by monocytes was observed, suggesting a relation between cholesterol and intracellular formation of pro-oxidant cytokines (Ferro et al., 2000).

1.5 PATHOGENESIS OF MYOCARDIAL INFARCTION

Acute Myocardial Infarction (AMI) generally occurs when coronary blood flow decreases abruptly after a thrombotic occlusion of a coronary artery previously narrowed by atherosclerosis. Slowly developing, high-grade coronary artery stenoses usually do not precipitate AMI because of the development of a rich collateral network over time. Instead, AMI occurs when a coronary artery thrombus develops rapidly at a site of vascular injury.

- In most of the cases, infarction occurs when an atherosclerotic plaque fissures, ruptures, or ulcerates and when conditions favour thrombogenesis, so that a mural thrombus forms at the site of rupture and leads to coronary artery occlusion.
- Histological studies indicate that the coronary plaques prone to rupture are those with a rich lipid core and a thin fibrous cap.
• Platelets are exposed to subendocardial collagen and necrotic plaque contents, leading to adhesion, aggregation, activation and release of adenosine-di-phosphate (ADP), a potent platelet aggregator, with build-up of a platelet mass. The platelet mass may give rise to emboli or potentiate occlusive thrombosis.

• Simultaneously tissue thromboplastin is released, activating the extrinsic pathway of coagulation.

• Adherent activated platelets release thromboxane A₂, serotonin, and platelet factors III and IV, predisposing to coagulation, favouring vasospasm, and adding to the bulk of the thrombus.

• Frequently within minutes, the thrombus evolves to become completely occlusive.

The schematic representation of the progression of myocardial necrosis after coronary artery occlusion are shown below in Figure 1.1

1.6 Transmural versus sub-endocardial infarction

Myocardial Infarction is of two types each having different morphology and clinical significance. The most common type is transmural infarct in which the ischemic necrosis involves the full or nearly full thickness of the ventricular wall in the distribution of a single coronary artery. This pattern of infarction is usually associated with coronary atherosclerosis, plaque rupture and super imposed thrombosis. In contrast, sub-endocardial infarct constitutes an area of ischemic necrosis limited to the inner one third or at most one-half of the ventricular walls, often extending laterally beyond the perfusion territory of a single coronary artery. The two types of infarcts are closely interrelated, because in experimental models and likely in humans, the transmural infarct begins with a zone of sub-endocardial necrosis that extends in a “wavefront” across the
full thickness of the thickness of the ventricular wall. Therefore, a subendocardial infarct can occur as a result of a plaque rupture, followed by coronary thrombus that becomes lysed before myocardial necrosis and extends across the major thickness of the wall.

In the past, an acute myocardial infarct diagnosed by enzyme elevation or other clinical criteria in which Q waves failed to develop on the electrocardiogram was considered a sub-endocardial infarct. The presence or absence of Q waves, however, does not reliably predict the distinction between transmural and sub-endocardial myocardial infarction with or without Q waves. The acute mortality, in patients which is half that in patients with Q wave infarcts have a risk of later infarction and a high late mortality rate. Thus, the non-Q wave infarct can be unstable rather than completely benign in condition.

1.7 **CLINICAL DIAGNOSIS OF MYOCARDIAL INFARCTION**

The clinical diagnosis of myocardial infarction is mainly based on three sets of data namely Symptoms, Electrocardiographic changes (ECG) and Elevations of specific serum enzymes.

1.7.1 **CLINICAL MANIFESTATIONS**

Typically, the onset is sudden and devastating with severe, substernal or precordial pain that often radiates to the left shoulder, arm, or jaw, often accompanied by sweating, nausea, vomiting, or breathlessness. Occasionally, the clinical manifestation consists only of burning substernal or epigastric discomfort that is misinterpreted as “indigestion” or “heartburn”. For about 10 to 15 % of patients, the onset is entirely asymptomatic and the disease is discovered only later by ECG changes.
Figure 1.1 Progression of myocardial necrosis after coronary artery occlusion.
1.7.2 **Electrocardiogram and Myocardial Infarction**

The ECG is usually a sensitive and specific way of confirming the diagnosis; however, it may be difficult to interpret, if there is bundle branch block or evidence of previous MI. The findings depend on several key factors such as the nature of the process [reversible (i.e., reversible) versus irreversible (i.e., infarction)], the duration (acute versus chronic), extent (transmural versus subendocardial) and localization (anterior versus inferoposterior), as well as the presence of other underlying abnormalities (ventricular hypertrophy, conduction defects).

Ischemia exerts 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. Such changes cause a voltage gradient between normal and ischemic zones. As a consequence, current flows between these regions. These so-called currents of injury are represented on the surface ECG by deviation of the ST segment.

The earliest ECG change is usually ST elevation; later on there is diminution in the size of the R wave and in transmural infarction a Q wave begins to develop. One explanation for the Q wave is that the myocardial infarct acts as an “electrical window”, transmitting the changes of potential from within the ventricular cavity and allowing the ECG to ‘see’ the reciprocal R wave from the other walls of the ventricle. Subsequently, the T wave becomes inverted because of a change in ventricular repolarisation; this change persists after the ST segment has returned to normal.

In contrast to transmural lesions, partial thickness or subendocardial infarction causes ST/T wave changes without Q waves or prominent ST elevation; this is often
accompanied by some loss of the R waves in the leads facing the infarct and is also known as non-Q wave or non-ST elevation myocardial infarction.

The ECG changes are best seen in the leads which ‘face’ the infarcted area. When there has been anteroseptal infarction, abnormalities are found in one or more leads from V1 to V4, while anterolateral infarction produces changes from V4 to V6, in aVL and in lead I. Inferior infarction is best shown in leads II, III and aVF, while at the same time leads I, aVL and the anterior chest leads may show ‘reciprocal’ changes of ST depression. Infarction of the posterior wall of the left ventricle does not cause ST elevation or Q waves in the standard leads, but can be diagnosed by the presence of reciprocal changes i.e. ST depression and a tall R wave in leads V1 to V4. Some infarctions also involve the right ventricle this may be identified by recording from additional leads placed over the right praecordium.

From a clinical viewpoint, the division of acute myocardial infarction into ST segment elevation and non-ST elevation (NSTEMI) types is useful since the efficacy of acute reperfusion therapy is limited to the former group.

1.7.3 CARDIAC MARKERS IN MYOCARDIAL INFARCTION

Certain proteins called serum cardiac markers are released into the blood in large quantities from the necrotic heart muscle after AMI. The rate of liberation of protein differs depending on their intracellular location and molecular weight and the local blood and lymphatic flow. The temporal pattern of protein release is of diagnostic importance, but contemporary urgent reperfusion strategies necessitate making a decision. Rapid whole blood bedside assays of serum cardiac markers are now available and may facilitate management decisions, particularly in patients with non-diagnostic ECGs.
The biochemical markers that are most widely used in the detection of MI are creatine phosphokinase (CPK), a more sensitive and cardiospecific isoform of this enzyme CK-MB, and the cardiospecific proteins troponin T and I. The troponins are also released, to a minor degree, in unstable angina with minimal myocardial damage. Serial, usually daily estimations are particularly helpful because it is the change in plasma concentrations of these markers that is of diagnostic value.

CPK starts to rise within 4 to 6 hours, peaks at about 12 hours and falls to normal within 48 to 72 hours. CPK is also present in skeletal muscle, and a modest rise in CPK may sometimes due to intramuscular injection, vigorous physical exercise or, in old people particularly, a fall. Defibrillation causes a significant release of CPK but not CK-MB or troponins. The most sensitive markers of myocardial cell damage are the cardiac troponins T and I, which are released within 4-6 hours and remain elevated for up to 2 weeks.

The American College of Cardiology and the European Society of Cardiology have redefined MI as a typical rise in cardiac troponin T or I, or CK-MB, above the 99th centile for normal, with at least one of the following: ischemic symptoms, development of pathological Q waves on the ECG, ischemic ECG changes (ST depression or elevation) or coronary artery intervention. This definition therefore includes non-ST segment elevation and Q wave development.

1.8 ISCHEMIA INDUCED CELLULAR INJURY

1.8.1 REVERSIBLE INJURY

The first point of attack of hypoxia is the cell’s aerobic respiration that is oxidative phosphorylation by mitochondria (Riemer and Ideker, 1987). As the oxygen
tension within the cell decreases, there is loss of oxidative phosphorylation and the
generation of Adenosine tri-phosphate (ATP) slows down or stops. This loss of ATP, the
energy source has a widespread effect on many systems within the cell. Heart muscle
ceases to contract within 60 seconds of coronary occlusion. The decrease in cellular ATP
and associated increase in adenosine mono-phosphate (AMP) stimulate
phosphofructokinase and phosphorylase activities. This result’s in an increased rate of
anaerobic glycolysis designed to maintain the cell’s energy sources by generating ATP
from glycogen. Glycogen is thus rapidly depleted. ATP is also generated anaerobically
from creatine phosphate, through the action of the enzyme creatine kinase. Glycolysis
results in the accumulation of lactic acid and inorganic phosphates from the hydrolysis of
phosphate esters. This reduces the intracellular pH. At this early period, there is also early
clumping of nuclear chromatin, apparently caused by reduced pH.

ATP depletion is primarily responsible for acute cellular swelling one of the
earliest event in ischemic injury. This is caused by an impairment of cell volume
regulation by the plasma membrane. To balance this, sodium is maintained at a lower
intracellular than extracellular concentration by energy-dependent sodium pump i.e Na\(^+\)
K\(^+\)-ATPase, which also keeps the concentration of potassium significantly higher
intracellularly than extracellularly. Failure of this active transport, owing to diminished
ATP concentration and enhanced ATPase activity, causes sodium to accumulate
intracellularly with diffusion of potassium out of the cell. The net gain of solute is
accompanied by an isosmotic gain of water, cell swelling and dilution of the endoplasmic
reticulum. A second mechanism for cell swelling in ischemia is the increased intracellular
osmotic load engendered by the accumulation of catabolites, such as inorganic
phosphates, lactate and purine nucleotides. In polarized epithelia, such as those in proximal tubules of the kidney, loss of polarity in the distribution of membrane enzymes occurs early during ischemia, accounting for early changes in transport by such cells (Molitoris, 1991).

The next phenomenon to occur is detachment of ribosomes from the granular endoplasmic reticulum and dissociation of polysomes into monosomes, probably owing to disruption of the energy-dependent interactions between the membranes of the endoplasmic reticulum and its ribosomes. If hypoxia continues, other alterations take place and again, are reflections of increased membrane permeability and diminished mitochondrial function. Blebs may form at the cell surface and cells that posses microvilli begin to lose their normal microvillous structure. “Myelin figures” derived from plasma as well as organellar membranes may be seen within the cytoplasm or extracellularly. They are thought to result from dissociation of lipoproteins with unmasking of phosphatide groups, promoting the uptake and intercalation of water between the lamellar stacks of membranes. At this time, the mitochondria are usually swollen, owing to loss of volume control by these organelles; the endoplasmic reticulum remains dilated and the entire cell is markedly swollen, with increased concentration of potassium. Upto a certain point, all of these disturbances is reversible, if oxygenation is restored.

1.8.2 Irreversible Injury

If ischemia persists, irreversible injury ensures. There is no universally accepted biochemical explanation for the transition from reversible injury to cell death. Irreversible injury however, is associated morphologically with severe vacuolization of the mitochondria including their cristae, extensive damage to plasma membranes and
swelling of lysosomes. Large, flocculent, amorphous densities develop in the mitochondrial matrix. In the myocardium, these are indications of irreversible injury and can be seen as early as 30 to 40 minutes after ischemia. Massive influx of calcium into the cell then, occurs particularly, if the ischemic zone is reperfused. There is continued loss of proteins, enzymes, coenzymes and ribonucleic acids from the hyperpermeable membranes. The cells may also leak metabolites, which are vital for the reconstitution of ATP thus, further depleting net intracellular high-energy phosphates.

At this stage, injury to the lysosomal membranes occurs, followed by leakage of their enzymes into the cytoplasm and activation of their acid hydrolyases. Lysosomes contain RNAases, DNAases, proteases, phosphatases, glucosidases and cathepsins. Activation of these enzymes leads to enzymatic digestion of cell components evidenced by loss of ribonucleoprotein, deoxyribonucleoprotein and, glycogen and various nuclear changes described later. Although these changes have been traditionally ascribed to falling pH, more recent studies suggest that the early fall in pH as irreversible injury proceeds.

Following cell death, cell components are progressively degraded and, there is widespread leakage of cellular enzymes into the extracellular space and conversely, entry of extracellular macromolecules from the interstitial space into the dying cells. Finally, the dead cell may become replaced by large masses composed of phospholipid form of myelin figures. These are then, either phagocytosed by other cells or degraded further into fatty acids. Calcification of such fatty acid residues may occur with the formation of calcium soaps. The leakage of intracellular enzymes across the abnormally permeable plasma membrane, and into the serum, provides important clinical parameters of cell
death. Cardiac muscle containing glutamic-oxaloacetate transaminase (GOT), glutamic-pyruvate transaminase (GPT), lactate dehydrogenase (LDH) and, creatine phosphokinase (CPK) are elevated in serum, and particularly the isoenzymes specific for heart muscle are valuable clinical criteria of myocardial infarction, a locus of cell death in heart muscle.

1.8.3 MECHANISM OF IRREVERSIBLE INJURY

A great deal of evidence indicates that cell membrane damage is a central factor in the pathogenesis of irreversible cell injury. Loss of volume regulation, increased permeability to extracellular molecules and demonstrable plasma membrane ultrastructural defects occur in the earliest stages of irreversible injury. Several biochemical mechanisms may contribute to such membrane damage which is enumerated below (Buja et.al., 1993, Bonventre, 1993).

i). Progressive loss of Phospholipids

In some ischemic tissues, irreversible ischemic injury is associated with a marked decrease in the content of membrane phospholipids (Chien et.al., 1981). Normally, the turnover of membrane phospholipids is coupled to their re-synthesis. Degradation of membrane phospholipids involves the action of endogenous phospholipases (PLA$_2$), whose activation is calcium dependent. One explanation for phospholipid loss is increased phospholipids degradation due to activation of endogenous phospholipases. Increased cytosolic calcium concentration induced by ischemia, as well as Ca$^{2+}$ - independent factors, contribute to such phospholipase activation. Progressive phospholipid loss can also occur owing to decreased reacylation or denovo synthesis of
phospholipids because these reactions involve ATP-dependent steps as well as appropriate substrate availability.

ii) Cytoskeletal Abnormalities

Cytoskeletal filaments serve as anchors connecting the plasma membranes to the cell interior. Activation of proteases by increased cytosolic calcium may cause damage to the cytoskeleton. In the presence of cell swelling, this damage results in detachment of the cell membrane from the cytoskeleton, rendering the membrane susceptible to stretching and rupture. This has been postulated as a mechanism for membrane damage in ischemic myocardium and indeed, there is evidence of increased degradation of the intermediate filament protein vinculin in ischemic heart disease (Armstrong and Ganote, 1992).

iii. Reactive Oxygen Species

Reactive oxygen species (ROS) are partially reduced oxygen free radicals and are of highly toxic molecules that can cause injury to cell membranes and other cell constituents. Such free radicals are present at very low levels in myocardium during ischemia, but there is an increase in free radical production on restoration of blood flow. Reperfusion results in a paradoxical effect: an increase in damage called reperfusion injury. This injury can be reduced by antioxidants in some models of ischemia. Although reactive oxygen species in post-ischemic tissue can be derived from incomplete reduction of oxygen by mitochondria and production of superoxide ion by xanthine oxidase (from vascular endothelium), it is thought that most oxygen species are produced by polymorphonuclear leukocytes that infiltrate the site of ischemia during reperfusion (Menger et al., 1992). It must be emphasized that if reperfusion does not occur, lethal
Figure 1.2 Potential outcomes of reversible and irreversible ischemic injury to the myocardium.
ischemic injury still eventually ensues, but toxic oxygen species are probably not involved under these conditions. The effects of these ROS are widely ranging, but four reactions are particularly relevant to cell injury.

1. **Lipid peroxidation of membranes** – Free radicals in the presence of oxygen may cause peroxidation of lipids within plasma and organellar membranes. Unsaturated fatty acids of membrane lipids possess double bonds between some of the carbon atoms. Such bonds are vulnerable to attack by oxygen-derived free radicals, particularly by OH (Farber, 1994) The lipid radical interactions yield peroxides, which themselves are reactive species, initiating the subsequent reduction of another fatty acid. An autocatalytic chain reaction ensues (called propagation), resulting in extensive membrane, organellar, and cellular damage.

2. **Oxidative modification of proteins** – Free radicals promote sulfhydryl mediated cross-linking of such liable aminoacids methionine, histidine, cystine and lysine as well as cause fragmentation of polypeptide chains. Oxidative modification enhances degradation of critical enzymes by cytosolic neutral proteases (Stadtman, 1992.) raising havoc throughout the cell.

3. **Lesions in deoxyribonucleic acid** – Reactions with thymine in DNA produce single strand break in DNA and such DNA damage has been implicated both in cell killing and in eventual malignant transformation of cells. Mitochondrial DNA is also affected severely.

4. **Lipid breakdown products**

   The unesterified free fatty acids, acyl carnitine, lysophospholipids and catabolic products that are known to accumulate in ischemic cells are broken down as a result of
phospholipids degradation. They have a detergent like effect on membranes. They also either insert into the lipid bilayer of the membrane or exchange with membrane phospholipids, potentially causing changes in permeability and electrophysiological alterations.

iv. Loss of intracellular aminoacids

Addition of certain aminoacids, principally glycine and L-alanine, protects hypoxic cells from irreversible membrane damage in-vitro, suggesting that loss of such aminoacids which occurs in hypoxia predisposes to membrane structural injury (Venkatachalam and Weinberg, 1993). The mechanism of protection by glycine and L-alanine are however, unclear.

v. Calcium paradox and cellular necrosis

Whatever the mechanism of membrane injury, the resultant loss of membrane integrity causes further influx of calcium from the extracellular space. When, in addition the ischemic tissue is reperfused to some extent, as may occur in vivo, the scene is set for massive influx of calcium. Calcium is taken up avidly by mitochondria after reoxygenation and permanently poisons them, inhibits cellular enzymes, denatures protein and causes the cytologic alterations characteristic of coagulative necrosis (Farber, 1990).

1.9 ISOPROTERENOL INDUCED MODEL FOR MYOCARDIAL INFARCTION IN RODENTS

Isoproterenol (ISO) induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function (Grimm et al., 1998). Exposure of the heart to high concentration of catecholamines had been reported to result in the development of necrotic lesions in the
The myocardium of experimental animals (Knufman et al., 1987). The pathophysiological changes due to ISO induced MI in rats are comparable to those taking place in human Myocardial Infarction (Wexler and Greenberg, 1978).

Isoproterenol [1-(3’,4’-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] (ISO) a synthetic catecholamine, acts as a β adrenergic agonist and has been found to cause severe stress in the myocardium resulting in the depletion of energy reserve of cardiac muscle cells and causes complex biochemical and structural changes leading to cell damage and necrosis (Rona, 1985). Catecholamines rapidly undergo auto-oxidation and it has been suggested that the oxidative products of catecholamines are responsible for changes in the myocardium (Yates and Dhalla, 1975). Isoproterenol-induced myocardial necrosis also involves membrane permeability alterations that bring about loss of function and integrity of myocardial membranes (Todd et al., 1980).

1.10 TREATMENT OF MYOCARDIAL ISCHEMIA

Transient episode of myocardial ischemia is due to an imbalance in myocardial oxygen supply and demand that may result from an increase in myocardial oxygen demand, a decrease in myocardial oxygen supply, or sometimes from both. Among the pharmacological agents used in the treatment of myocardial infarction are nitrovasodilators, Ca\(^{2+}\) channel antagonists, β adrenergic receptor antagonists, and antiplatelet agents.

1.10.1 NITROVASODILATORS

Organic nitrates are polyol esters of nitric acid, whereas organic nitrites are esters of nitrous acid. Nitrate esters (-C-O-NO\(_2\)) and nitrite esters (-C-O-NO) are characterized by a sequence of carbon–oxygen–nitrogen, whereas nitro compounds possess carbon–
nitrogen bonds (C-NO₂). The organic nitrates and nitrites, collectively termed nitrovasodilators, must be reduced to produce the reactive free radical NO, the active principle of this class of compounds. Nitrites, organic nitrates, nitroso compounds, and a variety of other nitrogen oxide–containing substances (including nitroprusside) lead to NO formation. NO activates guanylyl cyclase, increases the cellular level of cGMP, activates PKG and, modulates the activities of PDEs 2, 3, and 5 in a variety of cell types. In smooth muscles, NO-mediated increase in intracellular cyclic GMP activate PKG, which leads to reduced phosphorylation of myosin light chain, reduced Ca²⁺ concentration in the cytosol and vasorelaxation. Although the soluble isoform of guanylyl cyclase remains the most extensively characterized target for NO, NO also forms specific adducts with thiol groups in proteins and with reduced glutathione to form nitrosothiol compounds with distinctive biological properties.

1.10.2 Ca²⁺ Channel Antagonists

Voltage-sensitive Ca²⁺ channels (L-type or slow channels) mediate the entry of extracellular Ca²⁺ into smooth muscle and, cardiac myocytes and sinoatrial (SA) and atrioventricular (AV) nodal cells in response to electrical depolarization. In both smooth muscle and cardiac myocytes, Ca²⁺ is a trigger for contraction. Ca²⁺ channel antagonists, also called Ca²⁺ entry blockers, inhibit Ca²⁺ channel function. In vascular smooth muscle, this leads to relaxation, especially in arterial beds. These drugs also may produce negative inotropic and chronotropic effects in the heart. The Ca²⁺ channel antagonists approved for clinical use in the U.S. have diverse chemical structures including phenylalkylamines, dihydropyridines, benzothiazepines, diphenylpiperazines and a diarylaminopropylamine.
There is no evidence that Ca\textsuperscript{2+} channel antagonists are beneficial in the early treatment or secondary prevention of acute MI. In several trials, higher doses of the short-acting formulation of the dihydropyridine nifedipine had a detrimental effect on mortality. Diltiazem and verapamil may reduce the incidence of reinfarction in patients with a first non-ST segment elevation infarction who are not candidates for a β adrenergic receptor antagonist, but β blockers remain the preferred drugs.

1.10.3 β ADRENERGIC RECEPTOR ANTAGONISTS

β adrenergic receptor antagonists are effective in reducing the severity and frequency of attacks of exertional angina and in improving survival in patients after an MI. In contrast, these agents are not useful and may actually exacerbate vasospastic angina. Most β adrenergic receptor antagonists are equally effective in the treatment of exertional angina. Timolol, metoprolol, atenolol and propranolol have been shown to exert cardioprotective effects. The effectiveness of β adrenergic receptor antagonists in the treatment of exertional angina is attributable primarily to a fall in myocardial O\textsubscript{2} consumption at rest and during exertion, although there is also some tendency for increased flow toward ischemic regions. The decrease in myocardial O\textsubscript{2} consumption is due to a negative chronotropic effect (particularly during exercise), a negative inotropic effect and a reduction in arterial blood pressure (particularly systolic pressure) during exercise. Not all actions of β adrenergic receptor antagonists are beneficial in all patients. The decrease in heart rate and contractility causes increase in the systolic ejection period and left ventricular end-diastolic volume; these alterations tend to increase O\textsubscript{2} consumption. However, the net effect of β adrenergic receptor blockade is usually to decrease myocardial O\textsubscript{2} consumption, particularly during exercise. Nevertheless, in
patients with limited cardiac reserve who are critically dependent on adrenergic stimulation, β adrenergic receptor blockade can profoundly decrease left ventricular function. β adrenergic receptor antagonists that lack intrinsic sympathomimetic activity improve mortality in MI. They should be given early and continued indefinitely in patients who can tolerate them.

1.10.4 ANTIPLATELET, ANTI-INTEGRIN AND ANTITHROMBOTIC AGENTS

Aspirin reduces the incidence of MI and death in patients with unstable angina. In addition, low doses of aspirin appear to reduce the incidence of MI in patients with chronic stable angina. Aspirin, given in doses of 160–325 mg at the onset of treatment, reduces mortality in patients presenting with unstable angina. The addition of clopidogrel to aspirin therapy reduces mortality in patients with acute coronary syndromes. Heparin, in its unfractionated form or as low-molecular-weight heparin, also reduces symptoms and prevents infarction in unstable angina. Thrombin inhibitors such as hirudin or bivalirudin, are being investigated; these agents directly inhibit even clot-bound thrombin, are not affected by circulating inhibitors, and function independently of antithrombin III. Thrombolytic agents, on the other hand, are of no benefit in unstable angina. Intravenous inhibitors of the platelet GPIIb/IIIa receptor (abciximab, tirofiban, and eptifibatide) are effective in preventing the complications of percutaneous coronary interventions and in the treatment of patients presenting with acute coronary syndromes.

Since the different categories of anti-anginal agents have different mechanisms of action, it has been suggested that combinations of these agents would allow the use of lower doses, increasing effectiveness and reducing the incidence of side effects. Despite
the predicted advantages, combination therapy rarely achieves this potential and may be accompanied by serious side effects.

1.11 NUTRITION AND ISCHEMIC HEART DISEASE

Several observational epidemiologic studies have also suggested that higher intake of fruits; vegetables and whole grains are related to a lower risk of coronary heart disease (Law and Morris, 1998) (Pereira et al., 2001). Various hypotheses have been suggested to explain these beneficial effects of increased consumption of vegetables and fruits. An attractive hypothesis is that vegetables and fruits contain compounds that have protective effects, independent of those of known nutrients and micronutrients.

Many observational studies have been carried out to establish the dietary antioxidant intake and progression of cardiovascular disease and they all suggested that antioxidants such as vitamin E, vitamin C, lycopene from tomato, flavonoids and phytosterols possesses an inverse relationship with mortality due to CVD (Stephens et al., 1996, Gotto, 2003, Kritchevsky et al., 1995, Nyyssonen et al., 1997, Hertog et al., 1993, Street et al., 1994, Kohlmeier et al., 1997, Hak et al., 20003 and Hallikainen et al., 2000). Hence, novel dietary antioxidants have been given much importance these days, as there is perception to have protection against various cardiovascular diseases.

As the term implies “antioxidant” refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Based on the ‘oxidation theory’ for CVD, dietary antioxidants have attracted considerable attention as agents that protect cells or molecules from oxidative stress. The primary mechanism of its cardioprotective effect is likely through its effectiveness in overcoming oxidative stress. Hence, most in vitro, controlled intervention, ex vivo and animal model studies are designed as to
determine the impact of dietary antioxidants against LDL oxidation, which plays a key role in the early atherogenic process and vascular endothelial dysfunction (Kaliora et al., 2006).

1.12 SCOPE OF THE STUDY

Supplementation of antioxidants has attracted large population, as they are believed to have preventive function against chronic illnesses including cardiovascular disease. In view of this point, various fruits, vegetables and medicinal plants that are used for years together are found to have very good amount of antioxidants such as polyphenols, phytosterols, alkaloids, carotenoids and certain antioxidant vitamins. However, there is very little information available about the dietary antioxidative status. Numerous clinical trials have been attempted to study the role of dietary antioxidants as a preventive factor for various cardiovascular diseases and fails to clarify.

Novel antioxidants offer an effective and safe means of countering some of the problems and bolstering the body’s defense against free radicals and cardiovascular diseases. Many food materials which are taken daily in normal life by the local populations contain these antioxidants. Among them Dioscorea bulbifera Linn. tuber, was a famine food used by the tribal population of India. Also, it is referred in Ayurvedic and Chinese system of medicine. It also contain this phytochemical antioxidants in them. However, there is very little evidence to prove its antioxidant property and in turn its cardioprotective potential. A detailed study on the phytochemical and biological activity of Dioscorea bulbifera Linn. and the active saponin present in the tuber may highlight the potential of this tuber. Moreover, this study would help in scientifically validating the traditional claim in our Indian System of Medicine.
1.13 **OBJECTIVES OF THE STUDY**

Based on the scope of the study the present study envisaged in addressing the following objectives

- To standardize the plant material for further use.
- To evaluate the biological activity of the various fractions of the plant material by *in vitro* assays.
- To identify the cardioprotective potential of the effect of the effective fraction.
- To evaluate the mechanism of action of the effective fraction to be considered as a functional food.