CHAPTER 1

REVIEW OF LITERATURE
Review of Literature

Healthy human life is always cardinal for human being starting from his birth to the end of life. The number of diseases minor to major plays a key role in disturbing the healthy human life. Along with the modernization as well as sophistication in the life, human health directly or indirectly bids the challenge to several diseases resulting sometime survival and sometime surrender to diseases.

1.1. CARDIOVASCULAR DISEASES
Cardiovascular diseases (CVDs) are emerging as major cause of death in India. According to World Health Report (2004), non-communicable diseases caused 6.08 millions deaths of which cardiovascular diseases caused 3.31 million deaths in high child, high adult mortality region of South East Asia in the year 2002. In India, non-communicable diseases caused 5.10 million deaths, of which cardiovascular diseases were responsible for 2.78 million deaths. Of the cardiovascular diseases, ischemic heart disease caused 1.51 million deaths and other deaths were attributed to cerebrovascular diseases, rheumatic heart disease, hypertensive heart disease and inflammatory heart disease (Saradha and Jhan, 2009). CVDs will be the largest cause of death and disability by 2020 in India. In 2020 AD, 2.6 million Indians are predicted to die due to coronary heart disease which constitutes 54.1% of all CVD deaths. Nearly half of these deaths are likely to occur in young and middle-aged individual (30-60 years). Currently, Indians experience CVD deaths at least a decade earlier than their counterparts in countries with established market economies.

The contributing factors for the growing burden of CVDs are increase in prevalence of cardiovascular risk factors, especially hypertension, dyslipidemia, diabetes, overweight or obesity, physical inactivity and tobacco use. It is an area where major health gains can be made through the implementation of primary care interventions and basic public health measures targeting diet, lifestyles and environment (Saradha and Jhan, 2009). According to World Health Organisation
data, 16.7 millions 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). Coronary heart disease (CHD) has assumed epidemic proportions in India. The disease is more prevalent in urban populations and there is a clear gradient in its prevalence from rural to semi urban to urban populations. The disease occurs at the younger age in Indian subjects compared to Western developed nations (Bonow et al, 2000). Although spectacular progress has been made in understanding the pathophysiology of cardiovascular diseases, the treatment as well as its continuous management still eludes cure. Further, the high incidence and the increasing cost of treatment confirm the importance of cardiovascular diseases as a major health problem. Table.1 shows the lost due to cardiovascular diseases in India (Anderson and Chu, 2007).

Table. 1: Estimated DALYs (in millions) lost due to cardiovascular diseases in India

<table>
<thead>
<tr>
<th>DALY</th>
<th>1990</th>
<th>2000</th>
<th>2010</th>
<th>2020</th>
</tr>
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<tbody>
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<td>Cardiovascular diseases</td>
<td>12.25 (M)</td>
<td>15.94 (M)</td>
<td>20.91 (M)</td>
<td>27.79 (M)</td>
</tr>
<tr>
<td></td>
<td>11.20 (W)</td>
<td>12.56 (W)</td>
<td>14.02 (W)</td>
<td>15.74 (W)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>5.60 (M)</td>
<td>7.67 (M)</td>
<td>10.46 (M)</td>
<td>14.36 (M)</td>
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<tr>
<td></td>
<td>4.53 (W)</td>
<td>5.55 (W)</td>
<td>6.55 (W)</td>
<td>7.66 (W)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>2.13 (M)</td>
<td>2.79 (M)</td>
<td>3.65 (M)</td>
<td>4.84 (M)</td>
</tr>
<tr>
<td></td>
<td>2.11 (W)</td>
<td>2.43 (W)</td>
<td>2.75 (W)</td>
<td>3.13 (W)</td>
</tr>
</tbody>
</table>

DALY: disability adjusted life years, M: Male and W: Women
1.2. ECONOMIC IMPACT
Significant resources are currently allocated to health care and the treatment of cardiovascular disease. Direct and indirect costs for cardiovascular disease were estimated at nearly 337 billion dollars for 2004 (AHA 2004a). As a comparison, the cost for cardiovascular disease is nearly 15% of the total yearly federal budget. At a time when health care costs are increasing significantly and benefits are stagnant or decreasing, the issue of cost has become a significant political and societal issue. The Global Burden of Diseases (GBD) study reported the estimated mortality from CHD in India at 1.6 million in the year 2000. Extrapolation of these numbers estimates the burden of CHD in India to be more than 32 million patients. There is a significant burden of CHD on healthcare systems. The admissions due to acute myocardial infarction (MI) are increasing in India. The economic costs of CHD are poorly understood. It is roughly calculated that annually India spends about Rs.100 billion as direct cost of treatment. The magnitude of indirect costs is unknown and could be another Rs.100 billion. The sum is equal to 0.8% of the Indian national gross product (Saradha and Jhan, 2009).

1.3. MYOCARDIAL INFARCTION
The coronary circulation is unique in that it is responsible for generating the arterial pressure that is required to perfuse the systemic circulation and yet, at the same time, has its own perfusion impeded during the systolic portion of the cardiac cycle. Because myocardial contraction is closely connected to coronary flow and oxygen delivery, the balance between oxygen supply and demand is critical determinant of the normal beat to beat function of the heart. When this relationship is acutely disrupted by diseases affecting coronary blood flow, the resulting imbalance can immediately precipitate a vicious cycle, whereby ischemia–induced contractile dysfunction precipitate hypotension and further myocardial ischemia. Acute myocardial infarction (AMI or MI), more commonly known as a heart attack, is a disease state that occurs when the blood supply to a
part of the heart is interrupted. The resulting ischemia or oxygen shortage causes damage and potential death of heart tissue. It is a medical emergency, and the leading cause of death for both men and women all over the world (Katz, 1991).

Important risk factors are a previous history of vascular disease such as atherosclerotic coronary heart disease and/or angina, a previous heart attack or stroke, any previous episodes of abnormal heart rhythms or syncope, older age—especially men over 40 and women over 50, smoking, excessive alcohol consumption, the abuse of certain illicit drugs, high triglyceride levels, high LDL ("Low-density lipoprotein") and low HDL ("High density lipoprotein"), diabetes, high blood pressure, obesity and chronically high levels of stress in certain persons (Ross, 1992; Williams, 1990).

1.3.1. Pathophysiology of Myocardial Infarction

The most common cause of heart attack is atherosclerosis. Atherosclerosis is the gradual buildup of cholesterol and fibrous tissue in plaques in the wall of coronary arteries, typically over decades. Blood stream column irregularities visible on angiographies reflect artery lumen narrowing as a result of decades of advancing atherosclerosis. Plaques can become unstable, rupture, and additionally promote a thrombus (blood clot) that occludes the artery; this can occur in minutes. When a severe enough plaque rupture occurs in the coronary vasculature, it leads to myocardial infarction (necrosis of downstream myocardium). The development of atherosclerotic plaque occurs over a period of years to decades. The initial vascular lesion leading to the development of atherosclerotic plaque is not known with certainty.

The two primary characteristics of the clinically symptomatic atherosclerotic plaque are a fibromuscular cap and an underlying lipid-rich core. Plaque erosion may occur because of the actions of metalloproteases and the release of other...
collagenases and proteases in the plaque, which result in thinning of the overlying fibromuscular cap (Davies and Thomas, 1985; Davies et al, 1989).

The action of proteases, in addition to hemodynamic forces applied to the arterial segment, can lead to a disruption of the endothelium and fissuring or rupture of the fibromuscular cap. The degree of disruption of the overlying endothelium can range from minor erosion to extensive fissuring, which results in an ulceration of the plaque. The loss of structural stability of a plaque often occurs at the juncture of the fibromuscular cap and the vessel wall, a site otherwise known as the plaque’s "shoulder region". Disruption of the endothelial surface can cause the formation of thrombus via platelet-mediated activation of the coagulation cascade. If a thrombus is large enough to occlude coronary blood flow completely for a sufficient period, MI can result (Richardson et al, 1989).

Fig. 1.1: Narrowing of coronary artery during myocardial infarction

1.3.2. Mechanisms of myocardial injury
The severity of MI is dependent on three factors: first the level of the occlusion in the coronary artery; second the length of time of the occlusion; and third the
presence or absence of collateral circulation. Generally, the more proximal the coronary occlusion, the more extensive the amount of myocardium at risk of necrosis. The larger the MI, the greater the chance of death because of a mechanical complication or pump failure. The longer the period of vessel occlusion, the greater the chances of irreversible myocardial damage distal to the occlusion.

Under normal aerobic conditions cardiac energy is derived from fatty acids, supplying 60% to 90% of the energy for adenosine triphosphate (ATP) synthesis. The rest of the energy (10%–40%) comes from oxidation of pyruvate formed from glycolysis and lactate oxidation. Sudden occlusion of a major branch of coronary artery shifts aerobic or mitochondrial metabolism to anaerobic glycolysis within seconds. Reduced aerobic ATP formation stimulates glycolysis and an increase in myocardial glucose uptake and glycogen breakdown. Decreased ATP inhibits Na⁺/K⁺-ATPase, increasing intracellular Na⁺ and Cl⁻ leading to cell swelling. Derangements in transport systems in the sarcolemma and sarcoplasmic reticulum increase cytosolic Ca²⁺, inducing activation of proteases and alterations in contractile proteins. Pyruvate is not readily oxidized in the mitochondria, leading to the production of lactate, a fall in intracellular pH, a reduction in contractile function, and a greater ATP requirement to maintain Ca²⁺ homeostasis (Stanley, 2001).

Ultrastructurally, reversibly injured myocytes are edematous and swollen from the osmotic overload. The cell size is increased with a decrease in the glycogen content (Jennings and Ganote, 1976; Jennings et al., 1975; Virmani et al., 1990). The myocyte fibrils are relaxed and thinned; I-bands are prominent secondary to no contracting ischemic myocytes (Jennings et al., 1995). The nuclei show mild condensation of chromatin at the nucleoplasm. The cell membrane (sarcolemma) is intact and no breaks can be identified. The mitochondria are swollen, with loss
of normal dense mitochondrial granules and incomplete clearing of the mitochondrial matrix, but without amorphous or granular flocculent densities. Irreversibly injured myocytes contain shrunken nuclei with marked chromatin margination. The two hallmarks of irreversible injury are cell membrane breaks and mitochondrial presence of small osmiophilic amorphous densities (Jennings et al., 1974). The densities are composed of lipid, denatured proteins, and calcium. Irreversible ischemic injury is characterized by various processes involving the sarcolemmal membrane, eventuating in its disruption and cell death.

Increased cytosolic Ca\textsuperscript{2+} and mitochondrial impairment cause phospholipase activation and release of lysophospholipids and free fatty acids, which are incorporated within the cell and damaged by peroxides and toxic oxygen species. Cleavage of anchoring cytoskeletal proteins and progressive increase in cell membrane permeability result in physical disruption and cell death (Buja, 2005). If impaired blood flow to the heart lasts long enough, it triggers a process called the ischemic cascade; the heart cells die (chiefly through necrosis) and do not grow back. A collagen scar forms in its place. Recent studies indicate that another form of cell death called apoptosis also plays a role in the process of tissue damage subsequent to myocardial infarction (Krijnen et al., 2002).

1.3.3. Risk Factors and Symptoms of MI

Risk factors for atherosclerosis are generally risk factors for myocardial infarction: including Older age, Male gender (Wilson et al., 1998), Cigarette smoking, hypercholesterolemia (more accurately hyperlipoproteinemia, especially high low density lipoprotein and low high density lipoprotein), Diabetes (with or without insulin resistance), High blood pressure, obesity (Yusuf et al., 2005), change in lifestyle (Jensen et al., 1991) and inflammation (Wilson et al., 2006). Women that use combined oral contraceptive pills have a modestly increased risk of MI,
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especially in the presence of other risk factors, such as smoking (Khader et al, 2003).

AMI is usually characterised by onset of chest pain often described as a sensation of tightness, pressure or squeezing. Pain radiates most often to the left arm, but may also radiate to the lower jaw, neck, right arm, back, and epigastrium, where it may mimic heartburn. Shortness of breath (dyspnea), diaphoresis (an excessive form of sweating), weakness, light-headedness, nausea, vomiting, and palpitations. Loss of consciousness and even sudden death can occur in MI. In women, chest pain may be less predictive of coronary ischemia than in men (McSweeney et al, 2003). Approximately half of all MI patients have experienced warning symptoms such as chest pain prior to the infarction and one fourth does not experienced chest pain or other symptoms before infarction (Kannel, 1986).

1.3.4. Diagnosis and Laboratory finding of MI

The classical World Health Organisation (WHO) criteria for the diagnosis of MI require that at least two of the following three elements be present: a history of ischemic type chest discomfort, evolutionary changes on serially obtained ECG tracings, and a rise and fall in serum cardiac markers (Tunstall-Pedoe et al, 1994).

**Physical Examination:** includes heart rate (vary from a marked bradycardia to a rapid regular or irregular tachycardia, depending on the degree of left ventricular failure), blood pressure, temperature and respiration, jugular venous pulse, carotid pulse and Chest examination (wheezing, cough with hemaoptysis, pulmonary embolism with infarction).

**Cardiac examination:** includes palpitation, heart sounds, murmurs, friction rubs, funduscopic examination (provides information concerning the underlying vascular status), abdominal examination and neuropsychiatric findings (depends on patient mental status).
**Electrocardiography:** analysis of the constellation of ECG leads showing ST elevation may be useful for identifying the sites of occlusion in infarct myocardium.

**Serum markers of cardiac damage:** the increased use of more sensitive biomarkers of MI combined with more precise imaging technique has necessitated establishment of new criteria for MI. A cardiac specific marker includes Creatine kinase (CK), Creatine kinase isoenzymes (CK-MB), Myoglobin, Cardiac specific Troponins (TnT and TnI). Other laboratory finding includes Lipid profile, hematological parameters (white blood cell count, erythrocyte sediment rate) and marker of cardiac inflammation i.e. C-reactive proteins.

**Myocardium imaging:** Roentgenography, Ecocardiography, Computed tomography, Magnetic resonance imaging and nuclear imaging can be used for the experimental and clinical assessment of myocardial infarction (Libby *et al*, 2008).

1.3.5. Treatment of MI

A heart attack is a medical emergency which demands both immediate attention and activation of the emergency medical services.

1.3.5.1. First line treatment

Oxygen, aspirin, glyceryl trinitrate (nitroglycerin) and analgesics are administered as soon as possible. Morphine is the preferred pain relief drug due to its ability to dilate blood vessels, which aids in blood flow to the heart as well as its pain relief properties. Of the first line agents, only aspirin has been proven to decrease mortality. The ultimate goal of the management in acute phase of the disease is to salvage as much myocardium as possible and restore contractile function of heart chambers. This is achieved primarily by using thrombolytic drugs, such as streptokinase, urokinase, alteplase or reteplase.
Aspirin is a standard therapy that is part of all reperfusion regimens. Because irreversible ischemic injury occurs within 2-4 hours of the infarction, there is a limited window of time available for reperfusion to work. Although clinical trials suggest better outcomes, angioplasty via cardiac catheterization as first line measure is probably still underused. The goal of primary angioplasty is to open the artery within 90 min. of the presenting to the emergency room. If this time exceeds the time required to administer a thrombolytic agent by > 60 minutes, then the administration of the thrombolytic agents are preferred. Emergency coronary surgery, in the form of coronary artery bypass surgery is another option.

1.3.5.2. Secondary prevention

Patients are usually commenced on several long-term medications post-MI, with the aim of preventing secondary cardiovascular events such as further myocardial infarctions, congestive heart failure or cerebrovascular accident (CVA). Unless contraindicated, such medications may include.

Antiplatelet drug therapy such as aspirin and/or clopidogrel should be continued to reduce the risk of plaque rupture and recurrent MI. The combination of clopidogrel and aspirin may further reduce risk of cardiovascular events; however the risk of hemorrhage is increased.

Beta blocker therapy such as metoprolol or carvedilol should be commenced. These have been particularly beneficial in high-risk patients such as those with left ventricular dysfunction and/or continuing cardiac ischaemia.

ACE inhibitor should be commenced 24-48 hours post-MI in hemodynamically-stable patients, particularly in patients with a history of MI, diabetes mellitus, hypertension, anterior location of infarct (as assessed by ECG), and/or evidence of left ventricular dysfunction.
Statin therapy has been shown to reduce mortality and morbidity post-MI. Statins has plaque stabilization and multiple other ("pleiotropic") effects that may prevent myocardial infarction in addition to their effects on blood lipids.

Aldosterone antagonist agent eplerenone has been used in conjunction with standard therapies above. Omega-3 fatty acids, commonly found in fish, have been shown to reduce mortality post-MI.

1.4. REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS

Free radicals are generally unstable, highly reactive, and energized molecules. It exists with one or more unpaired electron(s) in atomic or molecular orbital. Free radicals are species containing one or more unpaired electrons in their outer atomic orbital. This electron imbalance renders them highly reactive and capable of widespread oxidation of lipids, proteins, DNA and carbohydrates. This eventually causes disruption of cell membranes, leading to release of cell contents and death (Halliwell, 1989). Reactive oxygen species in biological systems are related to free radicals, even though there are nonradical compounds in reactive oxygen species such as singlet oxygen and hydrogen peroxide.

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered nonradicals. Oxygen-centered radicals are superoxide anion, hydroxyl radical (·OH), alkoxyl radical (RO·), and peroxyl radical (ROO·).

Oxygen-centered nonradicals are hydrogen peroxide (H₂O₂) and singlet oxygen. Other reactive species are nitrogen species such as nitric oxide (NO·), nitric dioxide (NO₂·), and peroxynitrite (ONOO−) (Halliwell et al, 1989). Clinical studies reported that reactive oxygen species are associated with many age related degenerative diseases, including atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis and liver injury (Cohen et al, 2000).
Mitochondria, which consume more than 90% of the oxygen in aerobic living organisms, are the main source of reactive oxygen species and free radicals. Oxygen in mitochondria is reduced to water by 4 sequential steps (Ames et al, 1993). Perhydroxyl radical or its ionized form, superoxide anion, is the first reduced intermediate of oxygen. Hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (·OH) are inevitable intermediates from oxygen to water reduction steps in body. Approximately 1% to 5% of the oxygen consumed by mitochondria is reduced and converted to these reactive oxygen species (Ames et al, 1993). Harman (2000) suggested that initially generated superoxide anion and hydrogen peroxide are the main reactive oxygen species causing the oxidation of cells and tissues. Superoxide anion itself is not a strong oxidant, but it reacts with protons in water solution to form hydrogen peroxide, which can serve as a substrate for the generation of hydroxyl radicals and singlet oxygen (Stief, 2003).

1.4.1. Sources of Reactive Oxygen Species

Multiple sources of reactive oxygen species (ROS) have been identified in vascular cells. These include: Xanthine oxidase (Fleming et al, 2001), NADPH oxidase (Meyer et al, 1999; Hohler et al, 2000), and uncoupled NOS (Beretta et al, 2003; Landmesser et al, 2003). NADPH oxidase and uncoupled NOS are thought to be major contributors to intracellular O$_2$·. Additional ROS sources include the mitochondrial electron transport chain, lipoxygenase (Giardina et al, 1998), Glucose oxidase (al-Bekairi et al, 1994), and cytochrome P450 (Bai et al, 2001). The primary ROS produced by these oxidases is O$_2$·, except for xanthine oxidase which can produce both O$_2$· and H$_2$O$_2$, and glucose oxidase which directly produces H$_2$O$_2$ (Fig. 2). Another possible pathway for O$_2$· formation in endothelial cells is uncoupled NOS. The likelihood of NOS uncoupling varies with the cellular environment. Low or oxidized tetrahydrobiopterin (BH4) and/or low concentrations of L-arginine are associated with NOS decoupling (Kuzkaya et al, 2003; Ulker et al, 2003).
Previous reports demonstrate that exposure of vascular smooth muscle cells to H$_2$O$_2$ results in increased O$_2^-$ (Li et al., 2000). In addition, NOS contributes to endothelial cell O$_2^-$ formation upon exposure to native LDL (nLDL) (Stepp et al., 2002). Myeloperoxidase is an important source of ROS in the circulation and can result in more powerful radical formation including OH-, the most damaging ROS. Myeloperoxidase directly forms HOCl (Carr et al., 2000a) a strong acid used along with H$_2$O$_2$ and OH- to degrade invaders in the body as part of the immune system response including macrophages (Carr et al., 2000b). HOCl is formed via myeloperoxidase in circulation and vascular cells and is associated with macrophage oxidation of LDL (Eiserich et al., 2002).

![Graph: Sources of oxidants](oxidases, lipoxygenases, mitochondria, cytochrome P-450)

**Fig. 1.2: Generation of various oxidants by different sources**

### 1.4.2. Oxidative Stress and oxidants

Oxidative stress is associated with cardiovascular disease including atherosclerosis, hypertension, and diabetes mellitus (Griendling et al., 2003). Oxidative stress is described as an imbalance between anti-oxidant and pro-oxidant species or as the deregulation of prooxidants and anti-oxidants. The deregulation concept addresses the variable roles played by O$_2^-$ and H$_2$O$_2$ in vascular cells in both the endothelial cell basal state and the diseased state. O$_2^-$ and H$_2$O$_2$ plays a role in normal cellular
function and cellular signaling. Vascular cells maintain anti-oxidant mechanisms to reduce the impact of acute oxidative stress, scavenge ROS produced during normal cellular function and respiration, and to regulate aspects of cellular signaling via O$_2^-$ and H$_2$O$_2$. Reactive oxygen species (ROS), including hydrogen peroxide (H$_2$O$_2$), the hydroxyl radical (OH$^-$), superoxide anion (O$_2^-$), and the peroxynitrite (ONOOO$^-$) (Cai et al, 2003; Taniyama et al, 2003) contribute to the pro-oxidant/anti-oxidant imbalance.

In a non-diseased cardiovascular system, O$_2^-$, NO and other pro-oxidants and anti-oxidants are regulated. With onset of cardiovascular disease, the pro-oxidant/anti-oxidant balance is lost and pro-oxidants increase with an eventual decrease in anti-oxidant availability and antioxidant enzyme expression during chronic oxidative stress (Muller et al, 2004). The oxidant imbalance is normally associated with increased O$_2^-$ and decreased NO availability (Zhang et al, 2003). NO at elevated concentrations, also plays a role in the formation of additional RNS species, including NO$_2^-$ (Wolin 2002). While ROS and RNS are considered to be toxic, there is significant evidence to suggest that they are utilized in cellular signaling. This is especially true of H$_2$O$_2$, which has been implicated in multiple signaling cascades (Griendling et al, 2000).

Pro-oxidants

ROS and RNS are major pro-oxidants found within the vasculature. O$_2^-$, H$_2$O$_2$ and ONOO$^-$ are three of the key pro-oxidants. Additional pro-oxidants include OH$, HOCl$, and lipid radicals, which have all been shown to contribute to oxidative stress (Wassmann et al, 2004).

1.4.3. Molecular damage induced by free radicals

All the biological molecules present in our body are at risk of being attacked by free radicals. Such damage molecules can impair cell functions and even lead to cell death eventually leading to diseased state.
1.4.3.1. **Free radical damage to Lipid and Lipid peroxidation**

Membrane lipids when reacted with free radicals undergo the highly damaging chain reaction of lipid peroxidation (LPO) leading to production of large number of toxic byproducts and impairs the functioning of the cell. Initiation of LPO is due to the attack by any species, which can abstract a hydrogen atom from a methylene group i.e CH₂, leaving behind an unpaired electron on the carbon atom (·CH). The resultant carbon atom is stabilized by molecular rearrangement to produce a conjugated dienes, which then can react with an oxygen molecule to give a lipid peroxyl radical (LOO·). These radical can further abstract hydrogen atom from other lipid molecule to form lipid hydroperoxides (LOOH) and at the same time propagate LPO further. The process of LPO, gives rise to many products of toxicological interest like malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and various 2-alkenals.

1.4.3.2. **Free radical damage to Protein**

Oxidation of proteins can generate a range of stable as well as reactive products such as protein hydroperoxides that can generate additional radicals particularly upon interaction with transition metal ions. Some oxidized proteins that are functionally inactive can gradually accumulate with time and thereby contribute to the damage associated with ageing as well as various diseases.

1.4.3.3. **Free radical damage to DNA**

Free radicals such as 'OH and 'H react with DNA by adding to bases or abstraction of hydrogen atom from the sugar moiety. The C4-C5 double bond of pyrimidine is particularly sensitive to attack by 'OH, generating a spectrum of oxidative pyrimidine damage products, including thymine glycol, uracil glycol, urea residue, hydantoin, 5-hydroxydeoxyuridine and other compounds. Interaction of purine with 'OH can generate 8-hydroxydeoxyguanosine (8-OHdG) which has been
implicated in carcinogenesis and is considered as reliable marker for oxidative DNA damage.

1.5. REACTIVE OXYGEN SPECIES AND CARDIOVASCULAR SYSTEM

ROS and RNS have multiple roles within the cardiovascular system. At lower concentrations, ROS and RNS are important signaling molecules. ROS, including H$_2$O$_2$, also participate in pathway signaling related to cellular proliferation, migration, and apoptosis (Brown et al, 1999; Patel et al, 2000a). At higher concentrations, ROS and RNS participate in the alteration of cellular phenotype from a basal state to an activated state resulting in increased inflammatory signaling and in increased ROS and RNS formation.

The increase in ROS, RNS and inflammatory signaling results in increased leukocyte and platelet activation and increased leukocyte recruitment (Patel et al, 2000b; Stokes et al, 2002a; Stokes et al, 2002b). The modification of cellular phenotype and increased levels of ROS and RNS are associated with oxidative stress and vascular disease formation and progression. Increased pro-oxidants are associated with vascular disease and are thought to be an important early step in vascular disease development, including atherosclerosis and hypertension (Landmesser et al, 2003; Vassalle et al, 2004). Multiple pro-oxidants and anti-oxidants participate in the normal physiologic balance that is lost with oxidative stress.

In early atherosclerotic development, it has been proposed that endothelial cells contribute to ROS levels and ROS formation (Landmesser et al, 2003), while smooth muscle cells and fibroblasts are induced to form ROS after endothelial cell dysfunction (Harrison et al, 2003c). Previous studies have demonstrated increased vascular smooth muscle cell O$_2^-$ levels with H$_2$O$_2$ exposure. In addition, increase in systemic plasma H$_2$O$_2$ levels have been observed in patients with cardiovascular disease (Lacy et al, 1998).
Fig. 1.3: Metabolism of ROS and the mechanism of oxidative tissue damage leading to pathological conditions

Oxidative stress and increased ROS are associated with multiple cardiovascular disease states. Fig. 4 depicts several pathways by which ROS and RNS can mediate biological effects connected to the cardiovascular system. ATII binds a G-protein associated receptor, initiating a cascade of events that involves activation of O$_2^-$ production by the NAD (P) H oxidase NOX2. O$_2^-$ is converted by SOD into H$_2$O$_2$ and OH that mediates activation of MAPKs via a tyrosine kinase. MAPK activation can lead to cardiac hypertrophy or to apoptosis. The ROS that is generated can also signal through ASK-1 to induce cardiac hypertrophy, apoptosis or phosphorylate troponin T, an event that reduces myofilament sensitivity and cardiac contractility. Peroxynitrite radical thus form can cause lipid peroxidation, alter ion channel and its function. Catalase and glutathione reductase are shown as enzymatic pathways to produce water and oxygen from H$_2$O$_2$. 
The cardiovascular system is continuously exposed to both reactive oxygen and nitrogen species. Oxygen, although essential for tissue survival, can be injurious during reperfusion of previous ischemic myocardium. ROS may be formed by infiltration of white blood cells into ischemic myocardium or may be formed in the endothelial cells by the action of xanthin oxidase during the period of ischemia (McCord and Roy, 1982). Infiltration of activated immune cells (particularly neutrophils) into cardiac muscle is a potential mechanism of cardiac oxidant production. Clinical studies in cardiopulmonary bypass patients have demonstrated that neutrophil activation, leukocyte platelet aggregation and cytokine released from cardiac tissue are the primary contributors of acute inflammatory reaction during post-ischemic reperfusion (Zahler et al, 1999) suggesting the neutrophils play a key role in oxidant production and further accentuate cardiac dysfunction and remodeling.

**Fig.1. 4:** Mechanisms by which ROS can alter the structure and function of cardiac muscle
1.5.1. ROS and calcium ions

Oxidant stress caused by ischemia-reperfusion produces excessive intracellular calcium accumulation (Wu and Fehr, 1995). A high intracellular Ca\(^{2+}\) concentration can cause cellular damage in many different ways. This has been implicated as a primary event in irreversible myocardial injury and cell necrosis. Under oxidant stress, both low and high affinity Ca\(^{2+}\) binding activities were increased and a major amount of Ca\(^{2+}\) is associated with the membrane phospholipids. Since oxidants are known to promote the peroxidation of membrane phospholipids, it is likely that the changes in ATP independent Ca\(^{2+}\) binding by oxidants are due to alteration in the Phospholipid composition of the membrane.

Stimulation of either α or β adrenoreceptors has been reported to promote Ca\(^{2+}\) influx in the myocardium (Kaneko et al., 1995). An increase in the density of both receptors was observed in ischemic myocardium. Endogenous catecholamines were released during ischemia. It has been suggested that some of the excessive gain in Ca\(^{2+}\) during reperfusion involve influx of Ca\(^{2+}\) through pathways controlled by the adrenoreceptors. Actually, pretreatment with adrenoceptor blocking agents attenuated the Ca\(^{2+}\) gain induced by reperfusion (Tani, 1990). Oxidant induced increase in Ca\(^{2+}\) ion and the resultant stimulation of Ca\(^{2+}\) dependent protease(s) may be an important step in NF-κB activation.

1.5.2. ROS and lipotoxicity

Myocardial lipotoxicity refers to the accumulation of intra myocardial lipids concomitant with contractile dysfunction, often associated with myocyte death (Unger, 2000). Recently it was shown that lipid accumulation is a significant feature of clinical heart failure (Sharma, 2004). Lipid accumulation occurs when there is an imbalance between lipid uptake and β-oxidation, a circumstance that can occur via a variety of mechanisms. Lipid accumulation induces an increase in
the PPARα, a nuclear receptor that alters gene expression in response to lipids. PPARα increases fatty acid oxidation, and increased expression of PPARα has been associated with the development of cardiac dysfunction, including diabetic cardiomyopathy (Finck, 2003). Although the mechanism by which this occurs remains unclear, β-oxidation of fatty acids generates ROS, and data suggests that ROS play a role in the pathogenesis of PPARα-associated cardiomyopathies and lipotoxicity (Sharma, 2004).

1.5.3. Nitric Oxide and cardiac function

It has been shown that endothelial cells produce a factor, EDRF, which promotes vascular smooth muscle relaxation. Vascular endothelial cells contain an enzyme, nitric oxide synthase (NOS), which synthesizes NO (EDRF) which is labile free radical. Three major NOS isoforms have been identified. Two of these are constitutive and calcium and calmodulin dependent, nNOS primarily in neurons, and eNOS primarily in endothelial cells. The third isoform, iNOS, is inducible and calcium independent and is primarily involved in inflammation. Each of the three NOS isoforms converts l-arginine to l-citrulline and NO, and require the substrates NADPH and oxygen as well as the co-factor tetrahydrobiopterin (BH4) (Curtis and Pabla, 1997; Knowles and Moncada, 1994). During ischemia, the rise in myocardial calcium leads to initial activation, however, subsequently oxygen levels fall limiting its availability as a substrate. In addition, marked intracellular acidosis occurs with prolonged ischemia rendering the enzyme inactive. It has been suggested that alterations in NO formation in ischemic myocardium result in post-ischemic injury. It was shown that vascular reactivity is decreased in the post-ischemic heart (Masini et al, 2000) and inferred that this was due to altered NO production or breakdown; however, it was not known if NO generation was increased or decreased during and after myocardial ischemia.

Peroxynitrite (ONOO-) is formed through the interaction of NO and O₂⁻ (Guzik et al, 2002) with a reaction rate three times the rate of SOD reaction with O₂⁻. The
reaction to form ONOO⁻ is dependent upon equimolar concentrations of NO and O₂⁻. SOD scavenging of O₂⁻ is the dominant reaction until the intracellular concentrations of NO and O₂⁻ are roughly equivalent (Faraci et al., 2004). NO availability is reduced with elevated concentrations of O₂⁻, through the formation of ONOO⁻. ONOO⁻ has multiple effects on cellular signaling including inhibition of PGI₂ synthase (Zou et al., 1999), up regulation of PGH₂ via stimulation of cyclooxygenase, lipid peroxidation, NOS decoupling, DNA oxidation, cell damage and protein oxidation, (Radi et al., 2001), inhibition of mitochondrial respiration, and an overall reduction in intracellular nitric oxide availability (fig.5). Oxidation of proteins, DNA, and lipids are associated with increased levels of ONOO⁻, though ONOO⁻ oxidative action is reversible unlike that of OH⁻ (Virag et al., 2003).

![Diagram](image.png)

**Fig. 1.5: Effects of nitric oxide and peroxinitrite on cellular levels**

1.5.4. C-reactive protein (CRP)

C-reactive protein (CRP), a marker of inflammation, is an important predictor of future cardiovascular events in apparently healthy men and women (Shah, 2000; Libby et al., 2002) and could directly participate in the pathogenesis of
atherosclerosis through activation of endothelial cells (Verma et al., 2002a; Verma et al., 2002b). CRP, named for its capacity to bind to the C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described (Yeh and Palusinski, 2003). CRP, like other acute-phase proteins, is synthesized by the liver in response to microbial infection, tissue injury, and autoimmune disorders. It had been shown that interleukin-1β (IL-1β) and IL-6 strongly induced the expression of CRP in human hepatocytes and hepatoma cells.

Recently, human neuronal cells were found to produce CRP in Alzheimer's disease (Yasojima et al., 2000). In addition, renal cortical tubular epithelial cells were shown to produce CRP after inflammatory stimuli. Interestingly, CRP has also been found in human atherosclerotic plaques (Jabs et al., 2003), which could be the result of indirect deposit from circulating cells or direct production by cells in the arterial wall. Human coronary artery smooth muscle cells can synthesize CRP after stimulation by inflammatory cytokines (Paolo et al., 2003). CRP modulates the synthesis and release of Matrix Metalloprotenase-2 (MMP-2) mRNA expression and increase MMP-2 synthesis and release in vascular smooth muscle cell through mechanism involving activation of Mitogen-activated protein (MAP) kinase pathway. A number of studies have demonstrated an increased expression and activity of MMP-1, 2, 3 and 9 in human, rats and porcine heart after MI (Rohde et al., 1999).

1.5.5. Myeloperoxidase

Myeloperoxidase is a heme-containing enzyme that catalyzes the conversion of Cl- to the 2e-oxidant HOCl as the major reaction.

\[ \text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \rightarrow \text{HOCl} + \text{H}_2\text{O} \]

Myeloperoxidase is the only human enzyme known to generate HOCl, and chlorinated biomolecules are therefore considered specific markers of oxidation.
reactions catalyzed by the enzyme (Heinecke, 1999). The myeloperoxidase/H$_2$O$_2$/Cl$^-$ system can also give rise to 3-chlorotyrosine, chlorohydrins such as those of cholesterol and fatty acids, $\alpha$-chloro fatty acid aldehydes and free amino acid or protein bound tyrosyl radicals (Heinecke et al, 1993). Tyrosyl radicals themselves may participate in secondary oxidation reactions, including the oxidation of LDL (Savenkova et al, 1994). In addition, the myeloperoxidase/ H$_2$O$_2$/Cl$^-$ system or HOCl convert L-tyrosine into $\beta$-hydroxyphenylacetaldehyde that itself can react with the amino head group of phospholipids (Heller et al, 2000). The myeloperoxidase/ H$_2$O$_2$/Cl$^-$ system and HOCl also oxidize nitrite to the nonradical oxidant nitryl chloride (NO$_2$Cl) and the radical $^\cdot$NO$_2$, both of which promote nitration and can convert tyrosine into 3-nitrotyrosine (Eiserich et al, 1996). Recent studies provide evidence that myeloperoxidase, in fact, plays a major role in the generation of nitrating species in vivo and that formation of 3-nitrotyrosine is strictly dependent on the availability of $^\cdot$NO$_2$ (Kirsch et al, 2000).

1.5.6. Apoptosis

Apoptosis is a mechanism by which cells respond to damage triggering a program of cell death. Apoptosis has recently been recognized as a component of much common cardiac pathology including chronic heart failure, cardiac sudden death and ischemia-reperfusion injury (Gottlieb et al, 1994; Tanaka et al, 1994). In the heart, apoptosis is a dominant form of cardiac myocyte death in ischemia/reperfusion, which is well known to be associated with the production of ROS that exert harmful effects in this disorder (Singal et al, 1998). In human myocardial infarction, both apoptosis and necrosis have been observed, and apoptosis has been considered the predominant form of myocyte cell death in the border zone compared with noninfarcted and remote areas. It has been suggested that apoptosis is present in myocardial infarction and may play a role in ventricular dysfunction (Veinot et al, 1997; Saraste et al, 1997).
Many apoptotic stimuli in the heart have been recognized, including oxidative stress, serum withdrawal, angiotensin II, hyperglycemia, pressure overload, mitochondrial dysfunction, proapoptotic factors such as TNF-α, and loss of CM survival factors. The increased production of ROS activates the Bcl-2 family proteins, the mitochondrial pathway, caspases, and MAPKs in the heart and targets various intracellular proteins, leading to cellular hypertrophy, apoptosis, necrosis, and eventual heart dysfunction (Kumar and Jugdutt, 2003).

1.5.7. Role of Caspases in apoptosis

The caspase family consists of more than a dozen caspases (caspase 1 through caspase 14) required for the regulation of apoptosis and inflammation. (Van et al, 1997) Caspases have a common structure and are present in zymogen form, with an N-terminal prodomain large subunit and a C-terminal small subunit. Functionally, caspases are divided into 3 different types: apoptotic initiators (caspases 2, 8, 9, and 10), executioners (caspases 3, 6, and 7), and cytokine precursors (caspases 1, 4, 5, 11, 12, 13, and 14). In this oxidative stress-related caspase activation in the heart following events occurs.

Mitochondrial dysfunction causes release of cytochrome c which binds to Apaf-1 in the presence of ATP and further promotes activation of procaspase 9 and then to caspase 3. Activated caspase 8 promotes apoptosis by acting on Bcl-2-interacting domain, Bcl-2 family protein(s) and activating caspase 3, which starts apoptosis by way of cleavage of ICAD (inhibitor of caspase-activated DNase), PARP (poly(ADP-ribose) polymerase), protein kinase C-δ, and gelsolin (Van et al, 1997). These cleaved proteins induce both cytoplasmic and nuclear apoptosis, including DNA fragmentation. The broad-spectrum caspase inhibitor zVAD-fmk has been shown to inhibit apoptosis in an in vivo rat model of cardiac ischemia/reperfusion injury (Yaoita et al, 1998) (Fig.6).
1.6. CATECHOLAMINE INDUCED MYOCARDIAL NECROSIS

Both α- and β-adrenoceptors are present in the normal heart. Three types of β-adrenoceptors (β₁, β₂, and β₃) are considered to regulate cardiac function. The ratio of β₁- and β₂-adrenoceptors with respect to their densities in the heart is dependent upon the species and is usually modified in the diseased myocardium (Brodde and Michel, 1999). All β-adrenoceptors are coupled to adenylyl cyclase (AC) through G proteins; β₁- and β₂-adrenoceptors are coupled through stimulatory G (Gₛ) proteins. Alterations in the β-adrenergic signal transduction pathway are considered to cause cardiac dysfunction in various pathological conditions such as myocardial infarction (Ishigai et al, 1999), hypertensive heart failure (Lee, 1989), and pressure–volume overload cardiac hypertrophy (Sethi et al, 2007).

Fig. 1.6: Mechanism of myocardial dysfunction by apoptosis and necrosis
The involvement of differential changes in the β-adrenergic signal transduction pathway has also been documented in the left and right ventricles during the development of heart failure (Sethi et al, 2006). In rats with congestive heart failure due to coronary artery occlusion, a marked decrease in the number of β-adrenoceptors was found at early, moderate, and severe stages of disease (Dhalla et al, 1992). Catecholamines at low concentrations are considered to be beneficial in regulating heart function by exerting a positive inotropic effect; however, at high concentrations they produce deleterious actions. In fact, excessive release of catecholamines from the endogenous stores or administration of catecholamines at high doses may deplete the energy reserve of cardiomyocytes and thus may result in biochemical and structural changes for the development of irreversible damage.

![Fig. 1.7. Structure of Isoproterenol](image)

Isoproterenol (L-β-(3, 4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride), a sympathomimetics β-adrenergic receptor agonist, causes severe stress to the myocardium resulting in an infarct like necrosis of heart muscle (Sushma Kumari et al, 1989). The rat model of isoproterenol-(ISO) induced myocardial necrosis serves as a well accepted standardized model to evaluate several cardiac dysfunctions (Wexler, 1978) and to study the efficacy of cardioprotective agents (Rathore et al, 1998). The model of ISO induced myocardial infarction is widely used experimental model for several reasons. The model is characterized by an extraordinary technical simplicity, an excellent reproducibility as well as an acceptable low mortality (Grimm et al, 1998). Myocardial infarction 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 myocardial infarction (Nirmala and Puvanakrishnan, 1996).

ISO induced necrosis is maximal in the subendocardial region of the left ventrical and in the interventricular septum. These changes resemble the subendocardial laminar necrosis produced by myocardial ischemia in humans (Davies, 1977). 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).

1.6.1. Mechanisms of ISO induced myocardial infarction

Isoproterenol induced myocardial cell damage is a multifactorial process and several mechanisms for the cardiotoxic effects of high levels of isoproterenol have been suggested. These mechanisms include (a) functional hypoxia and ischemia, (b) coronary insufficiency, (c) alterations in metabolism, (d) decreased level of high-energy phosphate stores, (e) intracellular Ca²⁺ overload, (f) changes in electrolyte contents, and (g) oxidative stress. Although these changes represent individual pathological states, they are known to affect each other and thus are interpreted as complex entities (Fig.8).

Oxidative stress 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 cyclization. Adrenochrome (which results from the cyclization of epinephrine-o-quinone) can be oxidized to several other compounds such as adrenolutin, 5, 6-dihydroxy- 1-methylindole (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). According to Hawley et al.
(1967) the rate of internal cyclization of catecholamine-o-quinone to respective aminochrome varies for the different catecholamines. Isoproterenol has 20 times higher internal cyclization than norepinephrine. The aminochrome can also undergo further oxidation similarly to that shown for adrenochrome. In fact adrenochrome isomerizes to adrenolutin which can oxidize to 5, 6-dihydroxy-l-methylisatin (DHMIs) or to adrenochrome- adrenolutin dimer. All these oxidative reactions produce free radicals (Rupp et al., 1994).

The oxidized products have the ability to interact with sulphydryl groups of various proteins including enzymes (Bindoli et al., 1992) which results in changes in microsomal permeability and mitochondrial Ca²⁺ uptake as well as depressions in oxygen consumption and ATP production (Takeo et al., 1980; 1981). It also leads to production of superoxide anions and subsequently hydrogen peroxide, which in the presence of iron forms highly reactive hydroxyl radicals and cause protein, lipid, and DNA damage (Dhalla et al., 2000). Moreover, it has been shown that hydrogen peroxide in high concentrations can attenuate β-adrenoreceptor-linked signal transduction in the heart by changing the functions of Gs proteins and the catalytic subunit of the AC enzyme (Persad et al., 1998).

ISO produces a number of biochemical and electrophysiological alterations which precede the histological changes in the heart. The primary disturbances of ISO induced myocardial infarction has been reported to enhance adenyl cyclase activity, resulting in increased cAMP formation, which in turn would lead to the higher lipid accumulation in the myocardium (Subash et al., 1978). Several early events, such as ultrastructural changes (disorientation, rupture of myofilaments, mitochondrial swelling, and presence of abnormalities in the nuclear structure and plasma), 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 (Lehr, 1972).

Biochemical alterations in ISO-induced cardiomyopathy represent a complex of changes in glycogen, glucose, fatty acids, cholesterol, and triacylglycerol levels in the blood as well as in the myocardial tissue. The results of alterations in cardiac adenine nucleotides are somewhat contradictory; however Kako (1965) reported a decrease in ATP, ATP/ADP, and ATP/AMP ratios. On the other hand, a decrease
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in ATP and creatine phosphate stores indicates a decrease in the high-energy phosphate stores and a lowering of the energy state of the myocardium, which appear to be due to impairment in the process of energy production (Fleckenstein et al, 1974). Electrolyte changes in ISO-induced cardiomyopathy differ in the myocardial tissue and in the plasma or serum. In the myocardium, Na⁺ and Ca²⁺ contents have been reported to be increased, whereas all electrolyte levels in the serum were normal with the exception of Ca²⁺, which was low (Lehr, 1972) and changes including those in sarcolemma, sarcoplasmic reticulum and mitochondria, are mainly mediated by oxidative stress, which is known to result in alterations of enzyme activity and transport systems and cause disturbances in cellular homeostasis (Takeo et al, 1980; 1981).

Many of these systems have a biphasic character suggesting the occurrence of defensive or adaptive mechanisms. Early increases in the sarcolemmal and sarcoplasmic calcium pump mechanisms as well as late changes in mitochondrial calcium uptake are believed to help the myocardial cell in lowering the intracellular concentration of Ca²⁺; however, an early depression in sarcolemmal Na⁺/Ca²⁺ exchange and a late decrease in sarcolemmal and sarcoplasmic reticulum Ca²⁺ pump activities may contribute to the development of intracellular Ca²⁺ overload (Dhalla et al, 1991), which is known to produce myocardial cell damage and cardiac dysfunction. Lipolysis is one of the important determinants of ISO induced myocardial injury. Study also provides evidence that chronic β-AR stimulation markedly shows iNOS up-regulation, CRP release, and nitratative stress, and that iNOS-mediated nitratative stress functions as a main interface linking chronic β-AR activation and myocardial cell apoptosis (Aihua et al, 2006).

1.7. DEFENSES AGAINST OXIDATIVE STRESS

Antioxidants are substances that protect cells from damage caused by free radicals. Antioxidants interact with and stabilized free radicals and may prevent some of
the damage free radicals otherwise might cause. Anti-oxidant systems provide for
the regulation of pro-oxidants in cellular signaling and also balance the level of
pro-oxidants during acute oxidative stress. Superoxide dismutase, catalase, and
glutathione peroxidase are the primary anti-oxidants. There are additional non-
enzymatic anti-oxidants, including vitamins C and E. Vitamin C is taken up by
endothelial cells and stored as ascorbate to provide for $O_2^-$ scavenging within the
cell (Wasserman et al, 2004). Higher concentrations of pro-oxidants have also been
shown to inhibit and reduce the activity and expression of anti-oxidants, notably
MnSOD (MacMillan-Crow et al, 1999). This is of importance during vascular
disease where prooxidant regulation is lost and cells are exposed to chronic
oxidative stress.

1.7.1. Superoxide dismutase

The first enzyme involved in the antioxidant defense is the superoxide dismutase:
a metalloprotein found in both prokaryotic and eukaryotic cells (Fridovich, 1983;
Fridovich, 1986). The iron-containing (Fe-SOD) and the manganese-containing
(Mn-SOD) enzymes are characteristic of prokaryotes. In eukaryotic cells, the
predominant forms are the copper-containing enzyme and the zinc-containing
enzyme, located in the cytosol. The second type is the manganese containing SOD
found in the mitochondrial matrix (Fridovich, 1983). The biosynthesis of SOD is
mainly controlled by its substrate, the superoxide. Induction of SOD by increased
intracellular fluxes of $O_2^-$ has been observed in numerous microorganisms, as well
as in higher organisms (Crapo and McCord, 1976).

1.7.2. Glutathione peroxidase

Glutathione peroxidase catalyses the reaction of hydroperoxides with reduced
 glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction
product of the hydroperoxide by the oxidation of NADH to NAD+. This enzyme is
specific for its hydrogen donor, GSH, and nonspecific for the hydroperoxides
ranging from \( \text{H}_2\text{O}_2 \) to organic hydroperoxides. It is a seleno-enzyme; two-third of which (in liver) is present in the cytosol and one-third in the mitochondria (Faraci and Didion, 2004).

1.7.3 Catalase

Catalase present in almost all the mammalian cells is localized in the peroxisomes or the microperoxisomes. It is a hemoprotein and catalyses the decomposition of \( \text{H}_2\text{O}_2 \) to water and oxygen and thus protects the cell from oxidative damage by \( \text{H}_2\text{O}_2 \) and .OH (Wasserman and Topper, 2004).

![Diagram](image)

**Fig. 1.9: Pro-oxidant and Antioxidant Interactions**

The figure depicts the complex interactions that may begin with formation of superoxide from different vascular cell oxidases. It includes the formation of lipid free radicals (LOO·) and their scavenging of NO as well as NOS decoupling.
1.8 NATURAL ANTIOXIDANTS – A PROMISING RAY

Herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life (Berman, 2000). Leading the way in the new understanding is the discovery of herbs as potent free radical scavenger, antioxidants. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive. Therefore, in current years, substantial attention has been directed towards credentials of plants with antioxidant ability that may be used for human expenditure. The task of free radicals in many disease conditions has been well customary. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging critical bio-molecules (Pinn, 2000). In recent years one of the areas, which attracted a great deal of attention, is antioxidant in the control of degenerative diseases in which oxidative stress has been implicated.

In recent decades, substantial interest has been focused on antioxidant therapeutic strategies for cardiovascular disease. It is imperative to emphasize effective preventive strategies for the cardiovascular disease epidemic. In years past, vegetable, fruit and antioxidant-rich Mediterranean diets have been highlighted, since a number of epidemiological studies have shown a strong inverse relationship between cardiovascular disease and vegetable and fruit rich diets (Weisburger, 2002; Hu and Willett, 2002). The World Health Organization recommends 500 g of fresh fruits and vegetables per day (National Research Council, 1989). Antioxidant micronutrients have attracted special attention, particularly vitamin E, vitamin C, β-carotene and other carotenoids, such as lutin, zeaxanthin and lycopene, which have the greatest singlet oxygen-quenching properties (Halliwell and Gutteridge, 1989). More recently, there has been increased interest in putative dietary antioxidants like bioflavonoids, flavonals like quercetin or special phenol derivatives in red wine and oxygen-sensitive B
complexes, which are involved in the metabolism of homocysteine and L-arginine (Hirvonen, 2001; De Bree, 2002; Hinderliter and Caughey, 2003). Many epidemiological studies have shown some evidence of an association between cardiovascular disease and antioxidant intakes (Gey, 1990; Gey, 1989; Gaziano et al, 1990), but results have been conflicting.

1.8.1 Antioxidants in Isoproterenol induced MI

Several plant products having antioxidant activity have been tested against isoproterenol induced myocardial infarction. *T. chebula* pretreatment significantly normalized all mitochondrial enzymes and enzyme activity of tricarboxylic acid cycle (TCA). It also prevents lysosomal enzyme activity, it has been also reported to be a strong antioxidant and inhibit mitochondrial lipid peroxidation (Suchalatha et al, 2007). Aqueous leaf extract of *Azadirachta indica* A. Juss prevents haemodynamic, biochemical and histopathological changes (Peer et al, 2008). Garlic (*Allium stivum*, liliaceae) has reported to have variety of cardiovascular effects, including reduction in plasma cholesterol. Ingestion of garlic has resulted in hyperlipidemia and inhibits atherosclerosis. S-allyl cysteine sulphoxide (SACS) commonly called Alliin, is an organosulphur compound derived from garlic. Research on SACS showed it has protective effects against cardiac marker enzymes, lipids, lipoproteins, enzymes associated with lipid metabolism, lipid peroxides, antioxidants, and lysosomal enzymes in isoproterenol (ISO)-induced myocardial infarcted male Wistar rats (Sangeetha and Quine, 2006).

Ethanolic extracts of *Picrorrhiza kurroa* rhizomes and roots effectively reduces lipid metabolism in serum and heart tissue in male wistar rats (Subramaniam et al, 2001). Dietary supplement of *Cichorium intybus* protects lactate dehydrogenase activity in ageing myocardium during isoproterenol induced myocardial infarction. Department of pharmacology, All India Institute of Medical Sciences, New Delhi carried out extensive study on *Ocimum sanctum* (tulsi). *O. sanctum*
pretreatment augmented the basal endogenous antioxidants and restores the antioxidant status of the heart. (Sharma et al, 2001). Naringin is a predominant flavonone found in grape fruits and related citrus species, it is rapidly transpired into naringenin by the action of the enzymes such as alpha rhamnosidase and beta glucosidase. Naringin significantly decreased the levels of lipid peroxidation and improved antioxidant status, improved biochemical parameters, altered the level of troponin T. bands of lactate dehydrogenase LDH1 and LDH2-isoenzymes, ECG-patterns and lysosomal hydrolases in ISO model of rats (Rajdurani and Prince, 2006; 2007a; 2007b).

Aqueous extract of *C. longa* was found to be effective in restoring biochemical and histopathological markers of injury in myocardial necrosis, modulates collagen metabolism and histopathological alteration in ISO treated rats (Nirmala and Rengarajulu, 1999). Squalin shows cardioprotective effect by inhibition of lipid accumulation and its antioxidant properties (Kohno et al, 1995). Mangiferin is a pharmacologically active phytochemical and natural polyphenol antioxidant derivative present in the bark, fruits, roots and leaves of *Mangifera indica* Linn. Mangiferin maintain the lipid profile, antioxidant status in ISO induced rats (Nair and Devi, 2006). Ethanolic latex extract of *Calotropis procera* significantly reduces the elevated cardiac marker enzyme and histopathological alteration (Ahmed et al, 2004). Further Salvianolic acid, rutin (Mladenka et al, 2009), quercetin (Punithavathi and Prince, 2009), Curcumin trigonsus Roxb (Thippleswamy et al, 2009), Coconut water (Anurag and Rajamohan, 2003), Arjunolic acid (Sumitra et al, 2001), crocin of Crocus sativus (Goyal et al, 2009) also showed cardioprotective effects against ISO induced myocardial infarction by maintaining various marker enzymes, antioxidant status, ultra structural and histopathological alteration and haemodynamic parameters. Certain polyherbal formulation like Arogh (Suchalatha et al, 2004), DHC-1, an herbal formulation derived from the popular plants *Bacopa monniera*, *Emblica officinalis*, *Glycyrrhiza glabra*, *Mangifera indica*
and *Syzygium aromaticum* (Bafna and Balaraman, 2005), Activit and Khamira Abresham Hakim Arshad Wala a unani formulation (Goyal et al, 2009) have also shown a good cardioprotective effect against ISO induced cardiotoxicity.

**Why combination of antioxidants?**

Our health is our most important asset. As such, we need to protect and honor our bodies we live in and while here on this earth. One of the most effective means of protecting our health is by taking a complimentary combination of antioxidants. Studies have shown that antioxidants are uniquely different from one another and each may have the specific function in the body. However, they are also synergistic, and will work most effectively when they are used together. In the proper combination, they can perform a wide range of metabolic activities, free radical scavenging and preventative actions.

**There are important reasons why we should combine diverse antioxidants**

When all the necessary antioxidants spread throughout the body, they engage in the wide spectrum of metabolic activities simultaneously. Various antioxidants involved with their own unique activities assist each other in achieving a healthier body. In this manner, stress can be significantly reduced, thereby conserving energy and speeding up the elimination and healing processes.

Different antioxidants scavenge different type of free radicals so a combination of antioxidants may be more competent for protecting the body against free radical damage rather than using a single antioxidant.

Many antioxidants utilize other antioxidant nutrient that have been oxidized or used up while fighting the effects of free radicals.

Antioxidants are hydrophilic as well as lipophilic in nature. *In vivo* and *in vitro*, hydrophilic and lipophilic antioxidants work together in a network, recycling
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each other and thus creating an effective antioxidant defence system (Haramaki et al, 1998; Kagan et al, 1992; Van et al, 1995).

Many antioxidants protect other antioxidants from its autoxidation, or one antioxidant regenerate another antioxidant.

The synergistic activity can be produced by different cellular location of antioxidant (ex. β-carotene is more lipophilic than α-tocopherol, it will more likely be in the interior of the membrane).

1.9. VITAMIN E (α-tocopherol)

Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity. Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements. Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation. Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms, vitamin E might help to prevent or delay the chronic diseases associated with free radicals (Traber, 2006).

![Structure of Vitamin E (α-tocopherol)](image-url)

Fig. 1.10: Structure of Vitamin E (α-tocopherol)
Vit. E has a strong antioxidant activity, in addition to this it is involved in immune function, as shown primarily by in vitro studies of cells, cell signaling, regulation of gene expression, and other metabolic processes (Traber, 2006). Vit. E inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes. Vitamin E also increases the expression of two enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation. Many claims have been made about vitamin E’s potential to promote health and prevent and treat disease. The mechanisms by which vitamin E might provide this protection include its function as an antioxidant and its roles in anti-inflammatory processes, inhibition of platelet aggregation, and immune enhancement. A primary barrier to characterizing the roles of vitamin E in health is the lack of validated biomarkers for vitamin E intake and status to help relate intakes to valid predictors of clinical outcomes (Cannon et al., 1991; Devaraj et al., 1996; Ricciarelli et al., 1998; Freedman et al., 1996).

1.9.1. Vitamin E and cardiovascular health
Evidence that vitamin E could help, prevent or delay coronary heart disease (CHD) comes from several sources. The effect of dietary vitamin E has been examined in several studies, many of which have reported a clear association between the reduction in the relative risk of CVD with high intake or supplementation of vitamin E, although some have shown no such association. In vitro studies have found that Vit. E inhibits oxidation of low-density lipoprotein (LDL) cholesterol, thought to be a crucial initiating step for atherosclerosis. It also prevents the formation of blood clots that could lead to a heart attack or venous thromboembolism (Glynn et al., 2007). Prevention of oxidative modification of LDL by dietary vitamin E has been hypothesized as a plausible mechanism for its favorable effects in CVD. Cross-cultural studies in Europe reported that a higher
level of plasma vitamin E is associated with a lower mortality rate from CVD (Gey et al., 1993). Prospective cohort studies in men and women also reported a lower risk of CVD with long-term vitamin E supplementation (Stampfer et al., 1993). The reduced relative risk of death from heart disease has been reported also in elderly subjects who were supplemented with vitamin E (Losonczy et al., 1996).

An inverse association between CVD including angina or myocardial infarction with vitamin E supplement from a food source (Kushi et al., 1996) has been reported. High levels of vitamin E in RBC were associated with less thickening of the arterial wall in French patients. Hodis et al. (1995) reported less progression of coronary lesion when patients received vitamin E supplements. Supplementation with 400 or 800 IU/d natural vitamin E substantially reduced the rate of nonfatal myocardial infarction, with beneficial effects apparent after 1 yr. of supplementation (Stephens et al. 1996). Several observational studies have associated lower rates of heart disease with higher vitamin E intakes.

The Heart Outcomes Prevention Evaluation (HOPE) study, which followed almost 10,000 patients at high risk of heart attack or stroke for 4.5 years, found that participants taking 400 IU/day of natural vitamin E experienced no fewer cardiovascular events or hospitalizations for heart failure or chest pain than participants taking a placebo. Clinical trials have not provided evidence that routine use of vitamin E supplements prevents cardiovascular disease or reduces its morbidity and mortality. Some researchers have suggested that understanding the potential utility of vitamin E in preventing CHD might require longer studies in younger participants taking higher doses of the supplement (Blumberg and Frei, 2007).

1.10. GREEN TEA (*Camellia sinensis*)

Tea is second only to water as the most widely consumed beverage in the world. Green tea is derived from fresh tea leaves by steaming or drying at elevated
temperatures in a process that avoids oxidation of the polyphenolic compound. These compounds comprise up to 30% of the total dry weight of fresh tea leaves and include flavandiol, flavonoids, phenolic acids, and flavonols (commonly known as catechins). The four major green tea catechins are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and catechin (EC). During the manufacture of black tea, fully dried tea leaves are subjected to a full fermentation process, during which the polyphenolic compounds are extensively oxidized. Thus, catechins are reduced to only 3% to 10% of the remaining solids while bisflavanols, theaflavins, other oligomers, and thearubigins are formed, the latter accounting for more than 20% of the remaining solids (Yang, 1999).

Katiyar et al. (1994) found that EGCG, EGC, and ECG from green tea significantly inhibit Fe³⁺ or ADP-supported spontaneous lipid peroxidation in mouse epidermal microsomes. Each of these epicatechin derivatives (ECDs) was also effective in inhibiting photo enhanced LPO generated by incubating epidermal microsomes in the presence of photosensitizer, silicon phthalocyanine, and at 650 nm irradiation. At equimolar basis, EGCG, which is also the major constituent in GTP, showed maximum inhibitory effects compared to other ECDs. This study provides evidence for the antioxidative property of ECDs. Terao et al. (1994) also showed the antioxidative property of EC and ECG by measuring the inhibition of lipid

![Structure of Green tea catechin](image.png)

**Fig. 1.11: Structure of Green tea catechin**

### 1.10.1. Green tea and Free Radical Scavenging Activity

Katiyar et al. (1994) found that EGCG, EGC, and ECG from green tea significantly inhibit Fe³⁺ or ADP-supported spontaneous lipid peroxidation in mouse epidermal microsomes. Each of these epicatechin derivatives (ECDs) was also effective in inhibiting photo enhanced LPO generated by incubating epidermal microsomes in the presence of photosensitizer, silicon phthalocyanine, and at 650 nm irradiation. At equimolar basis, EGCG, which is also the major constituent in GTP, showed maximum inhibitory effects compared to other ECDs. This study provides evidence for the antioxidative property of ECDs. Terao et al. (1994) also showed the antioxidative property of EC and ECG by measuring the inhibition of lipid
peroxidation in large unilamellar liposomes composed of egg yolk phosphatidylcholine. This study provided the evidence that EC and ECG serve as powerful antioxidants against lipid peroxidation when phospholipid bilayers are exposed to aqueous oxygen radicals (Terao et al., 1994). Oral administration of green tea inhibited the formation of 8-hydroxydeoxyguanosine in mice and topically treated GTP inhibited hydrogen peroxide formation (Xu et al., 1992). Tea polyphenols may inhibit carcinogenesis through their antioxidative activities supported by finding that catechin inhibited NNK-induced DNA single-strand breaks in rat hepatocytes. Recently, Miller et al. (1996) reported the antioxidative properties of black tea polyphenols by investigating their abilities to scavenge free radicals in the aqueous and lipophilic phases. Tea polyphenols can also react with peroxyl radicals and thus terminate lipid peroxidation chain reactions.

1.10.2. Green Tea and Cardiovascular health

There is emerging evidence that tea consumption may reduce the risk of heart disease. GT is proposed to be a dietary supplement in the prevention of cardiovascular diseases in which oxidative stress and proinflammation are the principal causes (Tipoe et al., 2007). Clinical trials employing putative intermediary indicators of the disease, particularly biomarkers of oxidative stress status, suggest tea polyphenols could play a very important role in the pathogenesis of heart disease (McKay and Blumberg, 2002). GT and its catechins may reduce the risk of coronary heart disease by lowering plasma levels of cholesterol and triglyceride. Studies indicate that green tea catechins particularly (-)-epigallocatechin gallate, interfere with the emulsification, digestion and micellar solubilization of lipids, the critical steps involved in the intestinal absorption of dietary fat, cholesterol and other lipids (Koo and Noh, 2007). Continuous ingestion of green tea catechin from an early age prevents the development of spontaneously hypertensive rats, probably by inhibiting the future development of high blood pressure at the later ages (Ikeda et al., 2007). EGCG improves endothelial function and insulin
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sensitivity, reduced blood pressure and protects against myocardial ischemia-reperfusion injury in spontaneously hypertensive rats (Potenza et al, 2007).

Catechin, like a nitric oxide donor, may have a therapeutic use as an NO-mediated vasorelaxant and may have an additional protective action in myocardial ischemia reperfusion induced injury (Hotta et al, 2006). Tea catechins with a galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats and also because postprandial hypertriacylglycerolemia is a risk factor for coronary heart disease, it is suggested that catechins with a galloyl moiety may prevent this disease (Ikeda et al, 2007). Administration of EGCG can protect isoproterenol induced myocardial infarction by maintaining biochemical and histological parameters (Devika and Prince, 2008a; 2008b).

Several additional epidemiologic studies have found that tea may protect against heart disease. The Boston Area Health Study found that consumption of 1 cup of tea per day reduced the risk of MI by 44% in 340 cases compared with age, sex, and community-matched non-tea drinking controls (Sesso et al, 1999). Similarly, the Scottish Heart Health Study, which involved more than 11,000 men and women followed for an average of 7.7 years, found a strong inverse correlation between tea consumption and all cause mortality, coronary death, or any major coronary event, including nonfatal MI or coronary artery surgery (Woodward and Pedoe, 1999). In clinical trials, however, evidence regarding the effects of tea consumption on LDL oxidation remains conflicting.

1.11. LYCOPENE

Lycopene belongs to the carotenoid family. It is a natural pigment that imparts red color to tomato, guava, rosehip, watermelon, and pink grapefruit. Tomatoes (especially deep-red fresh tomato fruits) and tomato products are considered the most important source of lycopene in the Western diet (Rao et al, 1998). Lycopene is an acyclic open chain polyene with 13 double bonds and a molecular formula of
C₄₀H₅₆. There are 11 conjugated double bonds arranged in a linear array, making it longer than any other carotenoid. The acyclic structure of lycopene causes symmetrical planarity and, therefore, lycopene has no vitamin A activity. Lycopene is more soluble in chloroform, benzene, and other organic solvents than in water. Lycopene is absorbed more efficiently by the human body after it has been heat processed into juice, sauce, paste, or ketchup (Gartner et al., 1997; Sies and Stahl, 1992).

![Fig. 1.12. Structure of lycopene](image)

Food processing may improve lycopene's bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix, and increases the surface area available for digestion also the isomeric form of lycopene may be altered from trans-isomers to cis-isomers by the temperature treatment which enhances absorption in the body (Shi and Le Maguer, 2000). Lycopene is fat-soluble, absorption into tissues improves when oil is added to the diet (Lee et al., 2000). Studies have indicated that lycopene is an effective antioxidant and free radical scavenger. Because of its high number of conjugated double bonds, lycopene exhibits twice the singlet-oxygen-quenching activity of β-carotene and ten times the activity of α-tocopherol (vitamin E) (DiMascio et al., 1989).

1.11.1. Lycopene and Free Radical Scavenging Activity

Lycopene has been shown to be one of the most efficient singlet oxygen quencher and peroxyl radical scavenger among all the carotenoids. Chemical quenching by
carotenoids contributes only 0.05% to the overall quenching of superoxide although this process is responsible for the final decomposition of carotenoids (photo-bleaching) (Sies and Stahl, 1995). The physical quenching mechanism for carotenoids involves the transfer of excess energy from singlet oxygen to the carotenoid's electron-rich structure. The carotenoid is transformed by this added energy into a “triplet” state, and then instead of further chemical reactions, it returns to the ground state by interaction with the surrounding solvent and loses the extra energy as heat. During this the carotenoid structure is unchanged and can be reused several times; under these conditions the same carotenoid molecule can continue to quench additional singlet oxygen molecules (Stahl and Sies, 2003).

Lycopene, with 11 conjugated and 2 nonconjugated double bonds, is the most effective quencher among the carotenoids (Krinsky, 1994), and is about 100-fold more effective than α-tocopherol.

At low oxygen tension carotenoids were found to scavenge peroxyl radical most efficiently. (Kennedy and Leibe, 1992). They react and scavenge radicals by obtaining its missing electron by removing an electron from another molecule or a second way is for the radical to add itself to another radical in order to pair up its single electron and form an adduct. In either case, the electron-rich character of carotenoids makes them attractive to radicals, thus sparing other cell components (lipids, proteins, DNA) from radical damage (Palozza and Krinsky, 1991).

According to the study by Miller et al. (1996), among the carotenoids, lycopene was the most efficient scavenger of the ABTS-radical (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) followed by cryptoxanthin, lutein, zeaxanthin, and β-carotene. They also showed that the radical scavenging effect of lycopene exceeded that of trolox, a water-soluble analog of vitamin E, by a factor of three. Because of potent antioxidant activity lycopene was found to reduced cisplatin and gentamycin induced nephrotoxicity (Yilmaz et al, 2006), Mutagenicity of saliva (Masahiro et al, 2003). Oral administration of lycopene can reverse cadmium
suppressed body weight and lipid peroxidation (Rencuzogullari and Erdogan, 2007).

1.11.2 Lycopene and cardiovascular health

A number of *in vitro* and animal studies have been carried out to investigate the role of β-carotene in the prevention of coronary heart diseases (CHD). *In vitro* studies shown that lycopene can protect native LDL from oxidation and can suppress cholesterol synthesis. Lycopene was found to reduce 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase, a rate limiting enzyme in cholesterol synthesis *in vitro* (Dugas *et al*, 1998; Fuhrman *et al*, 1997). An interesting non-antioxidant function of lycopene was shown *in vitro* and in humans. Fuhrman *et al* (1997) showed that the addition of lycopene to macrophage cell lines decreased cholesterol synthesis and increased LDL receptors. Incubation with lycopene *in vitro* resulted in a 73% decrease in cholesterol synthesis, which was greater than that achieved with β-carotene. Lycopene resulted in a 34% increase in LDL degradation in the cells themselves and approximately 110% increase in the removal of LDL from the circulation. Administration of fresh tomato and tomato juice to human subject decreased serum triglyceride levels and low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol was increased (Shen *et al*, 2007).

Several *in vivo* studies also supported the cardioprotective potential of Lycopene. n-hexane extract of tomato effectively protects adrenaline induced myocardial infarction by maintaining biochemical and histological alteration (Roksana and Nargis, 2008). Lycopene protects Adriamycin-induced cardiotoxicity; Doxorubicin induced cardiotoxicity (Karimi *et al*, 2005). Supplementation of 10 or 20 mg lycopene tomato powder/kg of diet to rats showed significant prevention of total lipids, total cholesterol, triglycerides, lipoprotein fractions and atherogenic index.
Lycopene also exerts an antiatherogenic effect by inhibiting the expression of inflammatory agents in hyperhomocysteinemic rats (Liu et al, 2007).

Morphologic analysis revealed that lycopene markedly reduced the formation of atherosclerotic plaques in the aorta compared with the situation in rabbits on a high-fat diet alone (Hu et al, 2008). Hung et al, 2008 provided that lycopene is able to inhibit TNF-alpha-induced NF-kappaB activation, ICAM-1 expression, and monocyte-endothelial interaction, suggesting an anti-inflammatory role of lycopene and possibly explaining in part why lycopene can prevent cardiovascular diseases. lycopene reduces macrophage foam cell formation induced by modified LDL by decreasing lipid synthesis and down-regulating the activity and expression of Scavenger receptor-A. However, these effects are accompanied by impaired secretion of the anti-inflammatory cytokine, IL10, suggesting that it may also have a concomitant pro-inflammatory effect (Napolitano et al, 2007). Bansal et al (2006) also reported that lycopene 1mg/kg dissolved in olive oil can protect myocardial injury after ischemia and reperfusion in Wistar rats.

1.12. BOTTLE GOURD (*Lagenaria Siceraria*)

*Lagenaria siceraria* commonly known as Bottle gourd Syn. Doodhi, syn Lauki (Hindi), Kadoo (Marathi) this is official in Ayurvedic Pharmacopoeia. It is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health (Rahaman, 2003).

1.12.1. Traditional Uses

*Lagenaria siceraria* fruits are traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion stings, alternative purgative, cooling effects. It cures pain, ulcers and fever and used for pectoral cough, asthma and other bronchial disorders-especially syrup prepared from the tender fruits (Nadkarni, 1992). The pulp of the fruit is considered cool,
diuretic, antibilious, and useful in coughs and as antidote to certain poisons (Van and Gericke, 2000).

1.12.2 Chemical constituents

The edible portion of fruits is fair source of ascorbic acid, beta carotene and good source of vitamin B complex, pectin dietary soluble fibers and contains highest source of choline level-a lipotropic factor. It is also good source of minerals and amino acids (Kirtikar 2001, Nadkarni, 1992). The fruit is reported to contain the triterpeniode cucurbitacins B, D, G, H and 22-deoxy cucurbitacin. The fruit juice contains beta glycosidedase-elasterase enzyme (Van and Gericke, 2000). Two sterols were identified and isolated from Petroleum ether fractions of ethanol extract of dried fruit pulp of *Lagenaria siceraria* namely Fucosterol and campesterol (Shirwaikar, 1996). HPLC analysis of extract of flowering plant of *Lagenaria siceraria* shows presence of flavone-C glycosides (Baoranoswka, 1994). The effect of semi purified dietary fibers isolated from the fruit of *Lagenaria siceraria* effects on fecal steroid excretion was reported (Sannoumaru and Shimizu, 1996). It is also reported to have content with more proportion of soluble dietary fibers (SDF) than insoluble fibers.

![Fruits of Lagenaria siceraria](image)
SDF are having profound effect in lowering serum cholesterol, which also reveals that the pectin is predominant component of soluble fibers in *Lagenaria siceraria* fruits (Chang, 1995). Small amount of unidentified mono- and di-caffeoylquinic acid derivative was detected. 30% inhibition of superoxide formation in xanthine and xantine oxidase medium by methanolic extract (500μg/ml) from fruit of *Lagenaria siceraria* is reported (Jiwjinda, 2002). The fruit also contains Lagenin: a novel ribosome inactivating protein (Wang, 2000). The seeds are considered to be of least importance and having prime role in the human nutrition due to encapsulation of innumerable phytochemicals, vitamins, minerals, amino acids along with saponin and essential fixed oils especially of unsaturated type. Ripe seeds are having a 45% yield of clear limbid oil. Seed oil which have cooling effect, and can be applied in migraine type headache. In many parts of China, 3 grams per day of this species (the report does not say what part of the plant) has been used as a single treatment for diabetes mellitus (Duke, 1985). A Triterpene Bryonolic acid an antiallergic compound was reported from callus culture of *Lagenaria siceraria* roots. The fruit juice contains β-glycosidase (elasterase) (Tabata et al, 1993).

1.12.3. Pharmacological Activities

A modern pharmacological study shows that *Lagenaria siceraria* fruit possesses various beneficial effects. Chloroform and alcoholic extract of *L. siceraria* showed significant effects in lowering total cholesterol, triglyceride and low density lipoproteins along with an increased in HDL level in triton induced hyperlipidemia in rats (Ghule et al, 2006a). Isolated constituents from *Langenaria siceraria* fruit juice extract namely LSN-I, LSN-II and LSN-III showed antihyperlipidemic activity against triton-X induced hyperlipidemia in rats (Mohale et al, 2008). *Langenaria siceraria* Stand. fruit juice extract (LSFJE) shows analgesic effect in acetic acid induced writhing and formalin induced pain in mice. It also showed anti-inflammatory activity against acute inflammatory models i.e. ethyl phenyl propionate-induced ear edema, carrageenan and arachidonic acid-
induced hind paw edema and also the albumin induced paw edema in rats (Ghule et al, 2006b). Vacuum dried extract and methanol extract of \textit{L. siceraria} fruit also shows diuretic activity (Ghule et al, 2006c). \textit{L. siceraria} fruit showed maximum antioxidant activity against \textit{in vitro} model using DPPH. The juice as such and its ten times dilution showed radical scavenging activity where as 100 and 1000 times diluted juice did not show any radical scavenging activity (Deshpande et al, 2007). Extract is also effective in CCL\textsubscript{4} induced liver damage where it maintained the level of endogenous antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and marker of lipid peroxidation to that of normal (Fard et al, 2008).

Gangwal et al shows the immunomodulatory effects of n-butanol soluble and ethyl acetate soluble fraction of successive methanolic extract of LSF in rats. Both the fractions significantly increases total WBC, neutrophils and lymphocytes count while insignificant changes were observed in monocytes, eosinophils and basophils count (Gangwal et al, 2008). Ethanol extract of LS also showed significant prevention in reduction of humoral immune response, cellular immune response and percent neutrophil adhere on in mice in the presence of chemical stressor i.e. Pyrogallol (Deshpande et al, 2008). Mixture of sterols and two flavonoids were isolated from n-butanol and ethyl acetate soluble fractions of successive methanol extract of \textit{L. siceraria} fruit identified as oleanolic acid (I), mixture of \ensuremath{\beta}\text{-sitosterol} (II) and campesterol (III), isoquercitrin (IV) and Kaempeferol (V). Compound I and IV significantly inhibited delayed type hypersensitivity response in rats compared to control group animals. They also increased rate of carbon clearance from the blood of mice indicating increased phagocytosis (Gangwal et al, 2009). Deshpande et al (2008) shows that, ethanolic extract of LS epicarp shows hepatoprotective activity by preventing elevated levels of serum glutamate oxaloacetate, serum glutamate pyruvate transaminase, alkaline phosphatase, bilirubin and histopathological alteration. The antihepatotoxic
activity of different fractions of the ethanolic extract of *L. siceraria* fruit were also showed by Gopalan et al, (1996).

The fruit powder of *L. siceraria* also showed good cardioprotective effects. The fruit was studied against Doxorubicin induced cardiotoxicity in rats at 200mg/kg, p.o for 18 days. L.S prevents the alteration in endogenous antioxidants (superoxide dismutase, reduced glutathione) and lipid peroxidation where as markers of cardiotoxicity i.e CK-MB and LDH were significantly reduced. Further the L.S powder also showed the protection against changes in ECG and histopathological alteration induced by doxorubicin (Fard *et al*, 2008). Ethanolic extract of *L. siceraria* fruits also showed increased in force of contraction and decrease in rate of contraction (from 66 to 44) in isolated frog heart when perfused with normal ringer solution (Deshpande *et al*, 2008).

1.13.POMEGRANATE (*Punica granatum*)

*Punica granatum* belongs to the family of Punicaceae, is commonly known as pomegranate, grenade, granats and punica apple. Punica granatum has been used extensively as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies (Ricci *et al*, 2006; Sanchez *et al*, 2008). Extracts of all parts of the fruit appear to have therapeutic properties. Current research seems to indicate the most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones are the principal constituents of the *Punica granatum* tree and fruit (Lansky *et al*, 2007).

Over the past few decades, scientific investigations have provided evidence for the antioxidant, anti-inflammatory and anticancer effects of the pomegranate, laying a credible basis for some of its traditional ethnomedical use (Adhami and Mukhtar, 2007). Juice and peels of pomegranate possess potent antioxidant properties and
Pomegranate peel extract (PPE) with an abundance of flavonoids and tannins has been shown to have a high antioxidant activity. PPE, through its antioxidant effects, protected the liver against oxidative injury and fibrosis induced by biliary obstruction in rats (Toklu et al, 2007). Pomegranate protects rat gastric mucosa from ethanol or aspirin toxicity (Khennouf et al, 1999) protection of neonatal rat brain from hypoxia (Loren et al, 2005) prevention of male rabbit erectile dysfunction (Azadzoi et al, 2005) were all attributed to its antioxidant effects (Bell and Hawthorne, 2008).

1.13.1. Pomegranate and Free Radical Scavenging Activity

Pomegranate extracts have been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation in animals (Rosenblat et al, 2006) and increase plasma antioxidant capacity in elderly humans (Guo et al, 2008). Studies in rats and mice confirmed the antioxidant properties of a pomegranate by-product (PBP) extract made from whole fruit minus the juice, showing a 19% reduction in oxidative stress in mouse peritoneal macrophages (MPM), a 42% decrease in cellular lipid peroxide content, and a 53% increase in reduced glutathione levels (Rosenblat et al, 2006).

Fig. 1.14: Fruits of Pomegranate (Punica granatum)
Study in rats with CCl₄-induced liver damage demonstrated pretreatment with a pomegranate peel extract (PPE) enhanced or maintained the free-radical scavenging activity of the hepatic enzymes catalase, super oxide dismutase, and peroxidase, and resulted in 54% reduction of lipid peroxidation values compared to controls (Chidambara et al., 2002). Research in humans has shown a juice made from pomegranate pulp (PPJ) has superior antioxidant capacity to apple juice. Using the FRAP assay (ferric reducing/antioxidant power), Guo et al. (2008) found 250 mL PPJ daily for four weeks given to healthy elderly subjects increased plasma antioxidant capacity from 1.33 mmol to 1.46 mmol. In addition, subjects consuming the PPJ exhibited significantly decreased plasma carbonyl content (a biomarker for oxidant/antioxidant barrier impairment in various inflammatory diseases) compared to subjects taking apple juice.

1.13.2. Pomegranate and Cardiovascular health

In a double-blind, randomized, placebo-controlled trial carried out on 39 patients showed decrease in angina episodes by 50 percent, reduction in myocardial ischemia and improved myocardial perfusion (as measured by stress-induced ischemia) after consuming pomegranate juice (Sumner et al., 2005). In vitro, animal, and human trials have examined the effects of various pomegranate constituents on prevention and attenuation of atherosclerosis. PJ proved, significantly enhancing NO's effect on cardiac endothelium even at 2,000-fold dilutions. PJ did not influence endothelial nitric oxide synthetase (eNOS) expression. PJ protect NO from free radical destruction and augment the antiproliferative action of NO on rat aortic smooth muscle cells (Kawaii and Lansky, 2004). Mechanisms associated with the anti-atherogenic effects of pomegranate in this study include increased mouse peritoneal macrophages (MPM) uptake of oxidized LDL, decreased lipid peroxidation, and decreased cholesterol levels (Rosenblat et al., 2006). A small clinical trial demonstrated PJ inhibits serum ACE and reduces systolic blood pressure in hypertensive patients (Aviram and Dornfeld, 2001).
PE was shown to activate peroxisome proliferator-activated receptor (PPAR-α), a cardiac transcription factor involved in myocardial energy production via fatty acid uptake and oxidation. PPAR-activation decreased cardiac uptake and circulation of lipids. Decreases in the content of tissue triglyceride and plasma total cholesterol were observed after treatment with PE for four weeks in Zucker diabetic rats (Huang et al, 2005).