Cardiovascular diseases

Cardiovascular diseases (CVD) refer to the class of diseases that involve the heart or blood vessels (arteries and vein). While the term technically refers to any disease that affects the cardiovascular system, it is usually used to refer to those related to atherosclerosis (arterial diseases). Most western countries face increasing rates of CVD. Each year, heart disease kills more Americans than cancer. Roughly in very sixty five seconds, an American dies as a result of a coronary event. Diseases of the heart alone cause by thirty percent of all deaths, with other diseases of cardiovascular system causing substantial further death and disability. Two out of three cardiac deaths occur without the diagnosis of CVD. Although a relatively new epidemic in India, it has quickly become a major health issue with deaths due to CVD expected to double during 1985 to 2015 (Gupta, 2007).

Myocardial Infarction

Myocardial infarction (MI) occurs when the blood supply to a part of the heart is interrupted. It is caused by a disruption in the vascular endothelium associated with an unstable atherosclerotic plaque that stimulates the formation of an intra-coronary thrombus which results in coronary artery blood flow occlusion. If such an occlusion persists long enough (20 to 40 minutes) irreversible myocardial cell damage and cell death will occur (Figure 1). Atherosclerosis is the gradual build-up of cholesterol and fibrous tissue in plaques in the wall of arteries. The most common triggering event is the disruption of an atherosclerotic plaque in an epicardial coronary artery which leads to a clotting cascade, sometimes
resulting in total occlusion of the artery. Plaque rupture with subsequent exposure of the basement membrane cause platelet aggregation, thrombus formation, fibrin accumulation, hemorrhage into the plaque, and varying degree of vasospasm. This can result in partial or complete occlusion of the vessel and subsequent myocardial ischemia. Total occlusion of the vessel for more than 4-6 hours results in irreversible myocardial necrosis, but reperfusion within this period can salvage the myocardium and reduce morbidity and mortality (Fenton et al., 2006). 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 (Figure 2). Studies have indicated that another form of cell death called apoptosis also plays a role in the process of tissue damage subsequent to MI. As a result, the patient's heart can be permanently damaged (Krijnen et al., 2002).

Figure-1. External view of human heart and clot formation
Atherosclerosis, plaque rupture and endothelial dysfunction can also stimulate the formation of a blood clot which in turn interrupts blood flow and leads to necrosis. Endothelial cells line the inner wall of coronary blood vessels. When these cells become disturbed, a piece of atherosclerotic plaque can dislodge and land in a narrow section of a coronary artery. When this happens, blood flow is blocked and can cause severe chest pain or even a heart attack (Figure-2). Endothelial dysfunction seems to be related to reduce the levels of nitric oxide. Normal levels and function of nitric oxide help the coronary blood vessels relax and dilate. When these vessels are relaxed, there is more blood flow to the heart. Oxidative stress reduces the availability and function of nitric oxide, thereby reducing the ability of the coronary blood vessels to relax.

The endothelium is often damaged around areas of coronary artery disease (CAD). The resulting deficit of antithrombotic factors such as thrombomodulin and prostacyclin enhances thrombus formation. In addition, the tendency of several platelet derived factors (e.g. TXA$_2$, 5-HT) to cause vasoconstriction is increased in the absence of endothelial-derived relaxing factors. This may promote the development of local vasospasm, which worsens coronary occlusion.

The degree of coronary occlusion and myocardial damage caused by plaque rupture probably depends on systemic catecholamine levels as well as local factors such as plaque location and morphology, the depth of plaque rupture and the extent in which coronary vasoconstriction occurs. Severe and prolonged ischemia produces a region of necrosis spanning the entire thickness of the myocardial wall. Such a transmural infarct usually causes ST segment elevation. Less severe and protracted ischemia can arise when:
• Coronary occlusion is followed by spontaneous reperfusion
• The infarct-related arteries are not completely occluded
• Occlusion is complete, but an existing collateral blood supply prevents complete ischemia
• The oxygen demand in the affected zone of myocardium is smaller

Under these conditions, the necrotic zone may be mainly limited to the subendocardium, typically causing non-ST segment elevation.

Figure 2. Occurrence of stable angina, sudden cardiac death and cardiac failure
Symptoms of myocardial infarction

Chest pain is the major symptom of MI. One may feel the pain in only one part of the body, or it may move from the chest to the arms, shoulder, neck, jaw, belly area, or back.
The pain can be severe or mild. It can feel like:

- A tight band around the chest
- Bad indigestion
- Something heavy sitting on the chest
- Squeezing or heavy pressure

The pain usually lasts longer than 20 minutes. Symptoms may also go away and come back.

**Other symptoms of myocardial infarction include:**

- Anxiety
- Cough
- Fainting
- Light headedness, dizziness
- Nausea or vomiting
- Palpitations (feeling like your heart is beating too fast)
- Shortness of breath
- Sweating, this may be extreme

Elderly people with diabetes mellitus and women may have little or no chest pain or they may experience unusual symptoms (shortness of breath, fatigue, weakness). Myocardial infarcted patients frequently feel suddenly ill. Women often experience different symptoms from men. The most common symptoms of MI in women include shortness of breath, weakness, feeling of indigestion and fatigue. Approximately one fourth of all MIs are silent without chest pain or other symptoms.
Introduction

Risk factors

Myocardial infarction is the leading cause of death for both men and women all over the world (Wilson et al., 1998). The following are some of the risk factors of MI.

- Hypercholesterolemia
- Hypertension
- Tobacco smoking
- Diabetes mellitus (with or without insulin)
- Male sex
- Family history
- Obesity
- Stress
- Hyper homocysteinemia

Many of these risk factors are modifiable; so many MI’s can be prevented by maintaining a healthier lifestyle. Physical activity is associated with a lower risk of MI (Jensen et al., 1991). Non-modifiable risk factors include age, sex and family history of an early MI (before the age of 60), which is thought of reflecting a genetic predisposition. Women who use combined oral contraceptive pills have a moderately increased risk of MI, especially in the presence of other risk factors, such as smoking (Khader et al., 2003).

High blood cholesterol (Hypercholesterolemia)

A high level of cholesterol in the blood is associated with an increased risk of MI because cholesterol is the major component of the plaques deposited in arterial walls.
High blood pressure

High blood pressure is a risk factor for developing MI. Both high systolic pressure (when the heart beats) and a high diastolic pressure (when the heart is at rest) increase the risk of MI.

Tobacco use (Smoking)

Tobacco and tobacco smoke contain chemicals that cause damage to blood vessel walls, accelerate the development of atherosclerosis and increase the risk of MI. Smoking promotes the formation of a new atherosclerotic lesion. It can trigger transient myocardial ischaemia in patients with established CVD and it is a potent risk factor for acute MI and for sudden cardiac death (Goldenberg et al., 2003).

Diabetes mellitus

Both type -I and type-2 diabetes mellitus are associated with accelerated atherosclerosis. Therefore, patients with diabetes mellitus are at risk for reduced blood flow to the legs, coronary heart disease, erectile dysfunction, and strokes at an earlier age than non-diabetic subjects. Patients with diabetes mellitus can lower their risk through rigorous control of their blood sugar levels, regular exercise, weight control, and proper diets. Seventy five percent of all diabetics die due to atherosclerotic CVD. The incidence of MI is 3-4 times higher in diabetics (Levy, 2006).

Male gender

The incidence of MI is higher in men than in women of all age groups. This gender difference in MI incidence, however, narrows with increasing age (Cotran et al., 1994).
Family history

A family history of premature CVD increases an individual’s risk of MI. The etiology of familial coronary events is multifactorial and includes other elements such as genetic components and acquired general health practices eg. smoking and high fat diets (Cotran et al., 1994).

Obesity

Extreme obesity is a well known risk factor for MI. Obesity may increase the risk of MI by promoting established risk factors such as hypertension, diabetes mellitus and dyslipidemia. It may also have a direct toxic effect on the myocardium (Kenchaiah and Ramachandran Vasan, 2003)

Diagnosis of myocardial infarction

A number of laboratory tests are available. No-one is completely sensitive and specific for MI, particularly in the hours following onset of symptoms. Timings are important and are correlated with patients’ symptoms, electrocardiogram (ECG), and angiographic studies.

Electrocardiogram

The primary purpose of ECG is to detect ischemia or acute coronary injury. The ST- segment elevation could be a result of ischemic mediated myocardial injury. ECG findings suggestive of MI are elevations of the ST- segments and changes in the T-waves. After MI, changes can often be seen on the ECG called Q –waves, representing scarred heart tissue. The QRS complex of normal ECG represents the sum of all electrical forces generated from the ventricular myocardium. As the occlusion continues, T wave inversion will be present when the necrosis is transmural and the electrically inert area produces an enlarged Q-wave (Schweitzer, 1990).
Cardiac diagnostic markers

Cardiac diagnostic markers or cardiac enzymes are proteins from cardiac tissue in the blood. These proteins are released into the bloodstream when damage to the heart occurs as in the case of MI. Laboratory tests that are often referred to as cardiac diagnostic markers include:

- Cardiac troponins (the most sensitive and specific test for MI)
- Creatine Kinase –MB (CK- MB)
- Lactate dehydrogenase (LDH)
- Myoglobin

Cardiac troponins

The current guidelines are generally in favour of troponins subunits I or T, which are very specific for the heart muscle and are thought to rise before permanent injury develops. The cardiac troponins T and I have been found to have excellent sensitivity and specificity of myocardial necrosis. These are released during MI from the cytosolic pool of the myocytes. Its subsequent release is prolonged with the degradation of actin and myosin filaments. Troponins have proven useful for the diagnosis and subsequent risk stratification of patients presenting with acute chest pain (Hetland and Dickstein, 1998). Serum cardiac troponins level was elevated in ISO induced myocardial infarcted rats (Marikannan and Darlin Quine, 2011a).
Table 1: Infarction distribution with ST-segment elevation in myocardial infarction and consequences.

<table>
<thead>
<tr>
<th>ST Elevation</th>
<th>Affected coronary artery</th>
<th>Area of damage</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_1$ through $V_4$</td>
<td>Left coronary artery: left anterior descending</td>
<td>Antero lateral heart wall septum</td>
<td>Left ventricular dysfunction: decreased carbon dioxide, congestive heart failure Left bundle-branch block Right-bundle branch block Left posterior fascicular block Infranodal block (2° or 3°)</td>
</tr>
<tr>
<td>$V_5$ through $V_6$, I, aVL</td>
<td>Left coronary artery: left circumplex branch</td>
<td>left lateral heart wall</td>
<td>Left ventricular dysfunction: decreased carbon dioxide, congestive heart failure, infranodal block (2° and 3°)</td>
</tr>
<tr>
<td>II,III, aVF, $V_4R$</td>
<td>Right coronary artery: posterior descending branch</td>
<td>Inferior heart wall right ventricle</td>
<td>Hypotension (particularly with nitroglycerine and morphine, which can decrease preload) Supranodal 1° heart block Atrial fibrillation/flutter, premature atrial contractions, infranodal block (2° and 3°), papillary rupture</td>
</tr>
<tr>
<td>$V_8$ and $V_9$ or ST Depressions in $V_1$ and $V_2$</td>
<td>90% Right coronary artery: posterior descending branch, 10% Left coronary artery: left circumflex branch (will see elevations in $V_5$ through $V_6$)</td>
<td>Posterior heart wall</td>
<td>Hypotension Supranodal 1° heart block infranodal block (2° and 3°) Atrial fibrillation/flutter, premature atrial contractions, Papillary rupture (mur mur)</td>
</tr>
</tbody>
</table>
Creatine kinase-MB

Creatine kinase has three enzymes: MM, MB, and BB. The MM fraction is present both in cardiac and skeletal muscle, but the MB fraction is much more specific for cardiac muscle. About 15 to 40% of CK in cardiac muscle is MB, while less than 2% in skeletal muscle is MB. The BB fraction found in the brain, bowel and bladder are not routinely measured. Thus, CK-MB is a very good marker of acute myocardial injury because of its excellent specificity and it rises in serum within 2-8 hours of the onset of acute MI. The CK-MB is also useful for diagnosis of reinfarction or extension MI because it begins to appear after a day, dissipating in 1 to 3 days, so that the subsequent elevations are indicative of another event. Increased activity of serum CK-MB in ischemic myocardium is related to decreased oxygen availability in the tissues (Nirmala and Puvanakrishnan, 1994). Stanely Mainzen Prince (2011a) reported an increase in the activity of serum CK-MB in ISO induced myocardial infarcted rats.

Lactate dehydrogenase isoenzymes

Measurement of LDH –isoenzymes are necessary for greater specificity for cardiac injury and non specific increase of total LDH in the serum will occur following tissue damage. LDH catalyzes the conversion of pyruvate to lactate. LDH-1 isoenzyme is normally found in the heart muscle and LDH-2 is found predominantly in blood. A high LDH -1level to LDH-2 suggests MI. It can be differentiated from other types of tissue damage, since the LDH – isoenzymes begin to rise in 12- 24 hours following MI and peaks in 2-3 days gradually dissipating in 5-24 days (Jaffe et al., 1996). Stanely Mainzen Prince (2011a)
reported increased intensities of LDH-1 and LDH-2 isoenzymes in the serum of ISO induced myocardial infarcted rats.

**Myoglobin**

Myoglobin is a protein found in skeletal and cardiac muscle, which binds to oxygen. It is a very sensitive indicator of muscle injury. The increase in myoglobin in the blood determines the size of an infarction. The levels of myoglobin are found to be elevated in experimentally induced myocardial infarction (Tipnis et al., 2000).

**Angiography**

In difficult cases or in situations where intervention to restore blood flow is appropriate, coronary angiography can be performed. A catheter is inserted into an artery (usually the femoral artery) and pushed to the vessels supplying the heart. A radio-opaque dye is administered through the catheter and the sequence of x-rays (fluoroscopy) is performed. If obstructed or narrowed arteries are identified, angioplasty can be applied as a therapeutic measure.

**Histopathology of heart**

Histopathological examination of heart may reveal infarction at autopsy. Under the microscope, MI presents as a circumscribed area of ischemic and coagulative necrosis.

**Free radicals**

Oxygen-derived free radicals and their contribution to tissue injury during myocardial ischemia are particular during the phase of reperfusion. Free radical mediated peroxidation of membrane phospholipids and consequent changes in membrane permeability appears to be the primary target responsible for
cardiotoxicity induced by ISO (Singal et al., 1981). Hearts exposed to free radicals during ischemia /reperfusion exhibit many features of injury including K⁺ loss, action potential duration shortening leading to loss of developed systolic force, the progressive rise in diastolic force, depressed high energy phosphate levels, changes in cellular metabolism and damage to mitochondria.

**The role of reactive oxygen species in normal physiology**

Reactive oxygen species (ROS) present a paradox in their biological function. They prevent diseases by assisting the immune system, mediating cell signaling and playing an essential role in apoptosis. On the other hand, they can damage important macromolecules in cells and may have a role in carcinogenesis and CVD.

The formation of ROS is a natural consequence of aerobic metabolism and is integral for maintaining tissue oxygen homeostasis. Oxygen homeostasis, the balance between oxidants and antioxidants is maintained through a natural series of reduction – oxidation (redox) reactions involving the transfer of electrons between two chemical species: compounds that lose electrons (oxidized) and those that gain electrons (reduced). When oxygen homeostasis is not maintained, the cellular environment becomes oxidatively stressed. Approximately, 1-3% of oxygen consumed by the body is converted into ROS. Three of the major ROS – super oxide radical, hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻) are normal metabolic byproducts that are generated continuously by the mitochondria in growing cells (McCord, 2000; Castro and Freeman, 2001; Lopaczynski and Zeisel, 2004). Other significant intracellular sources of ROS include microsomal cytochrome P₄₅₀ enzymes, flavoprotein oxidases and peroxisomal enzymes
Introduction

involved in fatty acid metabolism. Oxidative stress related enzymes include superoxide dismutases for eliminating the superoxide radical as well as catalase and glutathione peroxidase (GPx) for removing $H_2O_2$ and organic peroxides (McCord, 2000; Castro and Freeman, 2001).

Transient fluctuations of ROS levels influence the activity of signal transduction pathways leading to cell proliferation, or to apoptosis or necrosis depending on the damage and duration of ROS and also on the cell type. Typically, low doses of ROS can be mitogenic, whereas medium doses lead to temporary or permanent growth arrest and high doses usually result in cell death either by apoptosis or necrosis (Holbrook and Ikeyama, 2002).

**Reactive oxygen species in cardiovascular diseases**

Cardiovascular diseases, including atherosclerosis and vascular disorders comprise the leading causes of death in developing countries (Duval et al., 2003). Vascular insults such as those associated with cigarette smoking, diabetes mellitus, hypertension and hyperlipidemia can trigger an inflammatory response in vessels; atherosclerosis is generally accompanied by chronic low grade inflammation (Sullivan et al., 2000; Droge 2002). Establishment of this inflammatory state mediated in part by ROS, results in damage to vascular endothelial and smooth muscle cells. Endothelial dysfunction characterized by pathological changes in endothelial cells. Anticoagulant, anti–inflammatory and vasorelaxation properties promote recruitment of monocytes, macrophages, growth factors and cellular hypertrophy, all of which contribute to the formation of atherosclerotic plaques. ROS activity helps to drive many of these processes.
**Lipid oxidation**

One of the most well known participants in atherosclerotic plaque formation is oxidized low density lipoprotein (ox LDL) (Itabe, 2003). LDL is the major cholesterol carrier in plasma and elevated levels of circulating LDL are associated with increased risk for atherosclerosis; increased levels of oxLDL have been associated with hypertension in men (Frostegard et al., 2003). Vascular trauma generates high levels of both intracellular and extracellular ROS in the vasoculture, thus leading to fatty acid and lipid peroxidation, particularly under conditions of hyperlipidaemia (Alexander, 1995). Oxidized lipids can affect cell function by accumulating in the cell membrane, causing leakage of plasmollemma and interfering with the function of membrane bound receptors (Cai and Harrison, 2000). Additionally, the byproducts of lipid peroxidation, such as unsaturated aldehydes and other metabolites, have cytotoxic and mutagenic properties. oxLDL itself has a specific role in the pathogenesis of atherosclerosis. It is specially recognized and taken up by receptors called “scavenger receptors” that are expressed as macrophages infiltration sites of vascular inflammation. Uptake of oxLDL by macrophages results in the formation of lipid filled macrophages called “foam cells” which undergo apoptosis and contribute significantly to the architecture of atherosclerotic plaque (Kovanen, 1993). oxLDL effects on the vessel themselves include stimulation of cytokine and growth factor production and generation of endothelial dysfunction, including inhibition of endothelial cell vasodilator function, all of which contribute to atherosclerosis.
**Oxidative stress**

Oxidative stress is a state of imbalance between free radical production, in particular ROS and the ability of the organism to defend against them, leading to progressive damage. The elevation in lipid peroxidation products in the circulation and the lowered antioxidant defense system are considered as direct proof of oxidative stress.

**Lipid peroxidation**

An increased lipid peroxidation is an important underlying cause of the initiation of oxidative stress related various tissue injuries, cell death and further progression of many acute and chronic diseases. Lipid peroxidation has three stages: initiation, propagation and termination (Figure-4).

**Initiation**

Initiation refers to any reaction that increases the overall rate of lipid peroxidation. Initiation means that initial hydrogen atom abstraction i.e., first chain initiation. Species that can abstract first hydrogen atom include OH, alkoxy radical (RO), peroxyl radical (ROO) and possibly HO but not H2O or O2 (Gutteridge, 1982). Initiation of lipid peroxidation occurs when a radical species with significant oxidizing character such as OH removes a methylene carbon of unsaturated fatty acid (LH) in bulk lipid. The free radicals annihilate each other to terminate chain (Halliwell and Gutteridge, 1984).

**Propagation**

Carbon centred radicals, which are produced from both the initiation and propagation processes, undergo molecular rearrangements. Lipid peroxide radical
abstracts an allylic hydrogen atom from another lipid molecule such as adjacent polyunsaturated fatty acids (PUFAs), resulting in the lipid hydroperoxide (LOOH) and second lipid radical (L'). This second lipid radical can proceed through the same reactions as the first, generating additional LOOH. The LOO⁻ radicals are able to remove hydrogen atoms from another lipid molecule such as an adjacent fatty acid. This causes the propagation of lipid peroxidation. The carbon radical formed can further undergo oxygen addition reaction to species form another peroxyl radical and so the chain reaction of lipid peroxidation continues. The peroxy radical combines with the hydrogen atom it abstracts giving a LOOH (Halliwell and Gutteridge, 1984).

**Termination**

The termination event can be the result of any reaction with another radical, protein or compound that acts as a free radical trap, forming a stable end product. The hydroperoxide produced may undergo different reactions to terminate the lipid peroxidation process. They may be reduced to a hydroxy fatty acid or undergo cyclization to produce cyclic endoperoxides. These endoperoxides can start with the reactions to form several biologically active prostaglandins, thromboxanes and leukotrienes via the cyclooxygenase and lipoxygenase pathways. Non-enzymatic pathways lead to the formation of compounds such as isoprostanes, aldehydes and alkanes, which also have concentration dependent signaling of cytotoxic effect in vivo. The formation of these end products constitutes the terminal stage of lipid peroxidation. Since PUFAs can have a number of C-H bonds susceptible to free radical attack, several end products can be formed from each PUFAs during lipid peroxidation (Halliwell and Gutteridge, 1984).
Antioxidants

Cells have a formidable defense against oxidative damage, many of which may not be readily recognized as antioxidants. Antioxidants are exogenous in nature, which prevents the generation of toxic oxidants and inactivate them, thereby blocking the propagation of chain reaction produced by these oxidants (Rangan and Burkely, 1993). There are two kinds of antioxidant defense to counterbalance the oxidative stress: enzymatic and non-enzymatic antioxidants.

Enzymatic antioxidants

Free radical scavenging enzymes such as superoxide dismutase, catalase, GPx and glutathione reductase (GR) and glutathione –S- transferase (GST) are the first line of cellular defense against oxidative injury.

Superoxide dismutase

Superoxide dismutase is widely distributed in cells, which play an important role in protecting the cells against oxidative damage. It is a class of metalloprotein and is a free radical metabolizing enzyme catalyzing the dismutation of superoxide, an oxidative radical formed from different aerobic metabolism. The $O_2^-$ in the presence of superoxide dismutase, can be converted to the reactive intermediate, $H_2O_2$.

$$2O_2^- + 2H \xrightarrow{SOD} H_2O_2 + O_2$$
Decreased activity of superoxide dismutase was observed in the heart of ISO induced myocardial infarcted rats (Punithavathi and Stanely Mainzen Prince, 2011).

**Catalase**

Catalase is a tetrameric enzyme present in most of the cells. It is located in the subcellular organelles, peroxisomes and mitochondria of liver. It is a heme protein capable of protecting hemoglobin and other cell components by preventing accumulation of $\text{H}_2\text{O}_2$ and $\text{OH}^-$ (Irshad and Chaudhuri, 2002). In most cells particularly central nervous system, $\text{H}_2\text{O}_2$ is detoxified by catalase. Catalase limits the formation of free radicals as well as neutralizing them once formed. It eliminates $\text{H}_2\text{O}_2$ by catalyzing a reaction between two $\text{H}_2\text{O}_2$ molecules, resulting in the formation of $\text{H}_2\text{O}$ and $\text{O}_2$.

$$2\text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} 2\text{H}_2\text{O} + \text{O}_2$$

The activity of catalase was found to be lowered in the heart of ISO induced myocardial infarcted rats (Sangeetha and Darlin Quine, 2006).

**Glutathione peroxidase**

Glutathione peroxidase is an important antioxidant enzyme, which is located mainly within the cytosol of eukaryotic cells but may also occur intramitochondrially. It is a tetrameric protein of MW = 84,000 consisting of four identical subunits. Each subunit contains one selenocysteine residue, which represents the catalytic element of the enzyme, firmly integrated in the protein.
Figure 4. Mechanism of lipid peroxidation
backbone. GPx catalyses the reduction of $\text{H}_2\text{O}_2$ and hydroperoxide to nontoxic products. The reducing equivalents of glutathione are used as substrates to form oxidized glutathione (GSSG) (Bruce et al., 1982).

$$\text{ROOH} + 2\text{GSH} \xrightarrow{\text{GPx}} \text{GSSG} + \text{ROH} + \text{H}_2\text{O}$$

Decreased activity of heart GPx was observed in ISO-induced myocardial infarcted rats (Sangeetha and Darlin Quine, 2006).

**Glutathione reductase**

Glutathione reductase is a ubiquitous FAD containing enzyme. This enzyme catalyses the GSSG to two molecules of GSH.

$$\text{NADPH} + \text{GSSR} + \text{H}^+ \xrightarrow{\text{GR}} \text{NADP}^+ + 2 \text{GSH}$$

It enables several vital functions of the cell such as detoxification of free radicals and ROS as well as protein and DNA biosynthesis by maintaining high ratios of GSH/GSSG (Schirmer et al, 1989). The activity of GR was decreased in the heart of ISO induced myocardial infarcted rats (Sangeetha and Darlin Quine, 2006).

**Glutathione – S-transferases**

Glutathione-S-transferases are widely distributed family of enzymes that catalyse the conjugation of electrophilic hydrophobic compounds with GSH. Multiple isoforms of GST are essential in order for a vast variety of toxic compounds to be detoxified. GSTs are phase-II drug metabolizing enzymes that
are also responsible for the glutathione conjugation of a variety of xenobiotics such as carcinogens, therapeutic and highly reactive lipid peroxidation products (Wilce and Parker, 1994). Jayalakshmi and Niranjali Devaraj (2004) reported a decrease in the activity of GST in the heart of ISO-induced myocardial infarcted rats.

**Non-enzymatic antioxidants**

The non-enzymic antioxidants and other molecules with antioxidant property include GSH, vitamin-C, vitamin-E and ceruloplasmin (Halliwell, 1990). Generally, a wide variety of biological molecules that can act as antioxidants by promoting the process of radical scavenging, both quenching free radicals directly or indirectly.

**Reduced glutathione**

Reduced glutathione is an abundant intracellular free radical scavenger and antioxidant. It is a tripeptide (Glu-Cys-Gly) and is the low molecular weight thiol found in mammalian cells. It is present in GSH and oxidized forms and functions as a free radical scavenger in radical induced cellular damage. GSH may stabilize membrane structure by removing acyl peroxides formed by lipid peroxidation reactions.

\[
2 \text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GSH}} 2 \text{H}_2\text{O} + \text{GSSG}
\]

Decreased concentration of GSH was observed in the heart of ISO induced myocardial infarcted rats (Sangeetha and Darlin Quine 2006).
Ascorbic acid

Ascorbic acid (mostly present in biological system as ascorbate anion) has very active hydroxyl group and therefore it is a very efficient free radical scavenger. The level of vitamin-C is found to be lowered in ISO induced myocardial infarcted rats, (Marikannan and Darlin Quine, 2011b).

α-Tocopherol

α-tocopherol is a lipid soluble phenolic derivative, having a very active OH group, which is responsible for higher antioxidant capacity. It is the only soluble antioxidant in human blood plasma and erythrocytes. Decreased level of α-tocopherol is observed in ISO induced myocardial infarcted rats (Marikannan and Darlin Quine, 2011b).

Ceruloplasmin

Ceruloplasmin is the major copper- carrying protein in the blood, and in addition plays a role in iron metabolism. It is an important in the control of membrane lipid oxidation probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation. An elevated level of plasma ceruloplasmin has been shown to be a risk factor for CVD including atherosclerosis and MI. The level of plasma ceruloplasmin is found to be elevated in ISO induced myocardial infarcted rats (Suchalatha and Devi, 2005).

Mitochondrial dysfunction in myocardial infarction

Mitochondrial dysfunction plays a vital role in the pathogenesis of MI. During ischemia intracellular oxidant produced. Under normal physiological
conditions, oxygen is essential for mitochondrial oxidative phosphorylation reactions and for production of ATP. The potentially toxic species (i.e., ROS) are formed intracellularly during mitochondrial electron transport and are controlled by intracellular antioxidant defense. The lack of oxygen supply by either hypoxia (reduction or interruption of coronary blood flow) disrupts mitochondrial electron transport chain, resulting in accumulation of toxic metabolites, acidosis, ATP, depletion, intracellular Ca$^{2+}$ overload, mitochondrial membrane depolarization, matrix swelling and cell death (Lemasters et al., 1967). Several experimental hypoxia or ischemia models, both in vitro and in vivo have suggested that injury in the myocardium is caused by oxygen radicals from the mitochondrial electron transport (Vanden Hock et al., 1998). The generation of large amounts of ROS can overwhelm the intracellular antioxidant defense network causing activation of neutrophils, lipid peroxidation, protein modification and DNA breaks (Ambrosio and Tritto, 1996) (Figure 5).

The cardiomyocyte is a most energy demanding cell in the body, and is totally dependent on oxidative phosphorylation to supply the large amount of ATP required for beat-by-beat contraction and relaxation (Halestrap et al., 2007). Mitochondria are involved in the production and regulation of cellular bioenergetics supply in the form of ATP (Marín-García and Goldenthal, 2004). They are the main consumers of molecular oxygen in the cardiac cells, and play a vital role in molecular events leading to tissue damage occurring in ischaemia (Suchalatha et al., 2007). The extent of recovery of the myocardium can be improved if mitochondrial damage is prevented or reduced i.e., the protected mitochondria may shift the balance away from irreversible injury towards cell recovery.
The heart mitochondrial marker enzymes isocitrate dehydrogenase (ICDH), sorbital dehydrogenase (SDH), malate dehydrogenase (MDH) and α-ketoglutarate dehydrogenase (α-KGDH) catalyse the oxidation of several substrates through the TCA cycle, yielding reduced equivalents, which are channeled through the respiratory chain for ATP synthesis by oxidative phosphorylation. ICDH is the predominant enzyme in the heart (Plaut et al., 1983), and it is the only NADPH-producing enzyme for the regeneration of glutathione in mitochondria. The enhanced activity of ICDH can contribute to the regeneration of GSH in heart mitochondria, resulting in a markedly high resistance of the heart enzymes against oxidative stress. SDH is one of the important enzymes that regulate the production of ATP in mitochondria and it is sensitive to free radicals. MDH catalyses a reversible NAD$^+$ dependent-dehydrogenase reaction involved in central metabolism and redox homeostasis between organelle compartments.

*Figure 5. Production of free radicals by mitochondria*
The α-ketoglutarate dehydrogenase catalyzes the conversion of α-ketoglutarate to succinyl-CoA and NADH in mitochondria. ISO induced myocardial infarcted Wistar rats significantly decreased the activities of TCA cycle enzymes, (ICDH, SDH, MDH and α-KGDH) in the mitochondrial fraction of the heart (Stanely Mainzen Prince, 2013).

Calcium is a key second messenger in the regulation of mitochondrial functions. Mitochondrial Ca$^{2+}$ uptake stimulates mitochondrial nitric oxide production. Dedkova and Blatter, (2008) reported that nitric oxide modulates mitochondrial O$_2$ uptake, ROS generation and ATP production, and thus represents a crucial link for excitation-metabolism as well as excitation-contraction coupling in cardiac cells. The increased intracellular calcium level is a cause of cytotoxicity induced by ISO due to its increased production of ROS. Cardiac mitochondrial calcium was elevated in ISO-induced myocardial infarcted rats (Marikannan and Darlin Quine, 2012).

**Classes of drugs used in the treatment of myocardial infarction**

Types of cardiovascular drugs may be divided into groups depending upon their action or what they treat. The number of cardiovascular drugs and even the number of categories is extensive. Doctors may use a combination of drugs, or may try some, only to switch to other types that appear to work more effectively for an individual patient. For those taking cardiovascular medicines, it is always important to understand their purpose and have facts about each drug side effects and interactions. This is especially the case when a person must take more than one medication, since some drugs may have very significant interaction with others or
a combination of medications may result in more side effects. Treatment categories that might describe drug actions include statins, diuretics, anticoagulants, beta-blockers, digitalis drugs, vasodilators, calcium channel blockers and angiotensin – converting enzyme (ACE) inhibitors.

**Statins**

Statins are cholesterol lowering drugs. These are used to control cholesterol levels (eg. Atorvastatin).

**Diuretics**

Diuretics are cardiovascular drugs that help to reduce fluid retention. These may also reduce pressure, though they usually aren’t first line blood pressure medications. When the body is retaining fluid, though, can often make the heart harder, and the intent with using diuretics is to reduce heart work load (eg. Furosemide).

**Anticoagulants**

Anticoagulants lengthen the time it takes for blood to clot, which can prevent the formation of blood clots that might cause a stroke. People who have artificial valves, who have had a stroke, or who are at risk for MI, may need an anticoagulant like warfarin to minimize further risk.

**Anti-platelet drugs**

Anti-platelet drugs are preferred to anticoagulants, and simple ones include medication like aspirin. These also work to keep blood clots from forming, but through a different mechanism than most anticoagulants.
Beta blockers

Beta blockers control pressure and reduce chest pain associated with angina. The various beta blockers show heart beat that may control numerous heart disease symptoms which may reduce future risk of MI (eg. Metoprolol).

Digitalis

Digitalis stimulates the heart to beat more forcefully. Those who are suffering from arrhythmias may require this medication. Digitalis is also used when a person is in congestive heart failure.

Vasodilators

Vasodilators like beta blockers may reduce the work of heart and they are often prescribed to treat chest pain resulting from angina.

Calcium channel blockers

Calcium channel blockers are used in the treatment of some forms of angina and also to treat certain arrhythmias or hypertension (eg. Nifedipine).

Angiotensin- converting enzyme inhibitors

Angiotensin-converting enzyme inhibitors decrease some blood supply to the heart which reduces its work. Cardiovascular drugs that fall into this category might lower blood pressure and increase heart function (eg. Captopril).

Induction of myocardial infarction

Pharmacological induction of MI by subcutaneous injection of ISO in animal model like rats has been found to be convenient. By studying the
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biochemical alterations that take place in an animal model, it is possible to gain more insight into the mechanisms leading to the altered metabolic processes in human MI. For the induction of MI in animals, adriamycin, cyclophosphamide and ISO can be used. ISO induced MI serves as a well standardized and common model to study the beneficial effects of many drugs and cardiac function. Administration of ISO causes ischemic necrosis in rats, which closely resembles the histological damage in human MI (Wexler, 1978).

Isoproterenol

![Structure of isoproterenol](image)

Figure 6. Structure of isoproterenol

We considered ISO induced MI in rats as a model to study the preventive effects of diosmin. ISO, (RS)-4-[1-hydroxy-2-(isopropylamino) ethyl] benzene-1,2-diol, (Figure 6) (a sympathomimetic β-adrenergic receptor agonist catecholamine) causes oxidative stress, depletes the energy reserve and produce myocardial damage (Li et al., 2006) to the myocardium resulting in an infarct like necrosis of heart muscle (Murugesan et al., 2011). The ability of ISO induced hyperlipidaemia resembles with the hyperlipidaemia of human beings. In addition, it showed many morphological and metabolic aberrations in the heart tissue of the experimental animals similar to those observed in human MI (Geng et al., 2004). This model is
characterized by an extraordinary technical simplicity, an excellent reproducibility, as well as an acceptable low mortality (Grimm et al., 1998) (Figure 7).

**Mechanism of isoproterenol**

Numerous mechanisms for the cardiotoxic effects of higher doses of ISO include hypoxia, ischaemia, coronary insufficiency, alterations in cardiac oxidative metabolism, decreased level of high-energy phosphate stores, intracellular $\text{Ca}^{2+}$ overload, changes in electrolyte contents and oxidative stress. ISO produces electrophysiological alterations and biochemical and membrane changes, which precede the histological and ultra-structural changes in the heart within 48 hours after the injection of first administration of excessive ISO (Upaganlawar et al., 2011).

![Figure 7. Advantages and some mechanism of isoproterenol](image-url)
Oxidative stress is one of the main mechanisms through which catecholamines exert their toxic effects. Figure 8 explains the metabolism of ISO. Spontaneous oxidation of catecholamine results in the formation of catecholamine-o-quinones, which produce aminochromes through cyclization. Adrenochrome results from the cyclization of epinephrine-o-quinone can be oxidized to several other compounds such as adrenolutin, 5, 6-dihydroxy-1-methylindole or adrenochrome-adrenolutin dimer. All these redox reactions lead to the formation of free radicals. Consequently, catecholamine-o-quinones, aminochromes and the radical species resulting from the oxidation of catecholamine are thought to be involved in catecholamine-related toxicity (Dhalla et al., 1992). These oxidative reactions have been reported to cause increased free radicals (Rupp et al., 1994). The oxidized products of ISO have the ability to interact with sulphhydryl groups of different proteins, leading to the production of superoxide anions and subsequently $\text{H}_2\text{O}_2$. These mechanisms cause changes in microsomal permeability, mitochondrial $\text{Ca}^{2+}$ uptake, decrease in ATP production and the formation of highly reactive $\text{OH}^-$. These changes cause damage to proteins, lipids and DNA of cardiac tissues (Bindoli et al., 1992; Dhalla et al., 2010).

The $\beta$-adrenergic receptor signal transduction pathway is critical for rapid adjustments to increased cardiovascular demand (Vatner et al., 1999). An excessive $\beta$-adrenergic stimulation produces an increased activity of vagus nerve and causes tachycardia. Tachycardia produces high demand for oxygen. The increased workload and reduced oxygen supply induce oxidative stress and affect normal functions of the heart.
Lipolysis is one of the important determinants of ISO-induced MI. An increased free radical production from excessive ISO affects lipid bilayer and leads to lipid peroxidative reactions (Mohan and Bloom, 1999). This may affect the membrane integrity of cardiac cells and affects their contractility.

**Figure 8. Proposed mechanism of auto-oxidation of isoproterenol**  
*(Singal et al., 1983)*

Changes in the balance between free radicals and antioxidants (Chattopadhyay et al., 2003), disturbances in glycoprotein components and electrolytes in the blood as well as in the myocardial tissue (Padmanabhan et al., 2008) are the notable pathological changes of ISO induced MI model.
Isoproterenol affects the oxidative metabolism in mitochondria and affect cellular homeostasis (Shukla et al., 2000). ISO affects cardiac lysosomal stability and disturb the regular function of cardiomyocytes (Ravichandran et al., 1990). Inducible nitric oxide synthase-mediated nitrative stress functions as a main interface linking chronic β-adrenergic receptor activation and myocardial cell apoptosis (Hu et al., 2006). Figure 9 shows various pathological changes which have been reported previously by excessive dose of ISO administration in rats.

![Diagram showing various pathological changes](Image)

**Figure 9. Pathological changes of isoproterenol induced myocardial infarction**

**Flavanoids**
Flavonoids are low molecular weight polyphenolic phytochemicals, derived from secondary metabolism of plants and play a vital role in various biological processes. They exhibit diverse type of properties that are beneficial for human health via interacting with a number of cellular targets involved in critical cell signaling pathways in the body. Flavonoids are components of a wide variety of edible plants, fruits, vegetables and of beverages such as tea, coffee, beer and wine. The majority of metabolic diseases are speculated to originate from oxidative stress and it is therefore significant that recent studies have shown the positive effect of flavones on diseases related to oxidative stress, such as atherosclerosis, diabetes mellitus, cancer, Alzheimer’s disease, etc. Some of the flavones of natural origin like naringenin, Gingko flavone glycosides and synthetic origin like flavopiridol are presently available in the market.

**Flavones**

Flavones are present in fruits and vegetables which we consume inadvertently in our daily diet and they have a positive impact on our health without any major adverse effects.

**Chemistry of flavone**

Flavone is a class of flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). The molecular formula of flavone molecule is \( C_{15}H_{10}O_{2} \). It has a three-ring skeletons, C6-C3-C6, and the rings are referred to as A, C, and B-rings, respectively (Figure 10). Flavones have three functional groups, including hydroxy, carbonyl, and conjugated double bond; consequently they give typical reactions of all three functional groups.
Pharmacological actions of flavones

Flavones scaffold can be termed ‘skeleton key’ as it is an important core in many compounds acting at different targets to elicit varied pharmacological properties with various substitution patterns (Figure 10). It is the diversity of this structure that gives flavones wide range of biological activity. Due to the wide range of biological activities of flavones, their structure–activity relationships have generated interest among medicinal chemists, and this has culminated in the discovery of several lead molecules in numerous disease conditions.

Anti-oxidant effect

The high levels of free radicals in living systems are able to oxidize biomolecules, leading to tissue damage, cell death or various diseases such as cancer, CVD, arteriosclerosis, neural disorders, skin irritations and inflammations. Free radicals are highly reactive and therefore can attack membrane lipids, generating carbon radicals and produce peroxy radicals which cause lipid peroxidation. Therefore, a single radical may damage many molecules by initiating lipid peroxidation chain reactions. Chrysin, a flavone, exhibits antioxidant effect.
Anti-inflammatory effect

Inflammation has foremost role in several disease conditions like asthma, atherosclerosis, Alzheimer’s disease, rheumatoid arthritis, diabetes mellitus, carcinoma, Crohn’s disease, gout, multiple sclerosis, osteoarthritis, psoriasis, bacterial or viral infections (Grivennikov, 2010). Different inflammatory mediators involved in these conditions are plasma proteases, prostaglandins, leukotrienes, histamine, serotonin, nitric oxide, interleukins (IL-1 to IL-16), iNOS production, tumor necrosis factor-α (TNF-α), NF-κB and chemokines (Nathan 2002; Feghali and Wright 1997). These mediators are produced through diverse signaling pathways involving cyclooxygenases, caspases and kinases like cyclin dependent kinases (CDK1 and CDK5), mitogen activated protein kinase 38 (MAPK38), c-Jun N-terminal kinase (JNK), serine threonine kinases (IKK1 and IKK2), interleukin receptor associated kinase 4 (IRAK-4), Janus kinases (JAK1- JAK3) etc (Dinarello, 2010).

Natural flavones such as apigenin, luteolin as well as synthetic flavones have been reported to bind with various protein kinases directly and alter their phosphorylation state that regulates multiple cell signaling pathways. Thus, flavones can have excellent therapeutic value in the treatment of inflammatory and autoimmune diseases (Hou and Kumamoto, 2010).

Anti-microbial activity

Natural products have particularly been a rich source of anti-infective agents. Flavones have been reported for protective role against microbial invasion. In plants, flavones accumulate as phytoalexins in response to microbial attack. Due to this protective role, flavones have been used for many years in traditional medicine to treat infectious diseases (Cushnie and Lamb, 2005). Nitrogen
containing flavones have been reported to have considerable antimicrobial activity. The compounds bearing amino, alkyl, cyano or alkenyl, alkyl group on piperazine are found to be the potent antibacterial and antifungal agents. Various synthetic biflavones revealed anti-bacterial, anti-fungal and anti-viral activity. Considering the antifungal properties, amentoflavone, isocryptomerin, ginkgetin and bilobetin were shown to have good antifungal action (Jung et al., 2006; Lee et al., 2009; Isobe et al., 2003) (Figure 11).

**Cardioprotective effect**

Since ancient times, numerous flavonoids, including flavones (Apigenin) have been reported to reduce occurrence of various heart diseases like CVD, arrhythmias, atherosclerosis, hypertension, ischemic stroke, peripheral arteriopathy, congestive heart failure etc. Flavones attributed to an increase HDL-cholesterol levels, antioxidant capacity, lipid regulation, inhibition of platelet aggregation, improving endothelial function, and the anti-inflammatory effects (Friedman and Kimball, 1986). A natural flavone, luteolin-7-O-β-D-glucopyranoside exhibited cardioprotective effects against doxorubicin-induced toxicity in H9c2 cells, reduced LDH and CK level, and decreased the elevated intracellular concentration of ROS and $[\text{Ca}^{2+}]$. The effects of flavone on myocardial post-ischemic-reperfusion recovery were reported and found that flavone treatment caused better recovery of left ventricular developed pressure (Ning et al., 1993).

Oxypropanolamine substituted flavones were shown to exhibit antihypertensive activity in spontaneously hypertensive rats. In cardiac disorders, abnormal rhythm of the heart increases the risk of death, congestive heart failure,
and heart strokes. Atrial fibrillation is the most common form of cardiac dysrhythmia. Acacetin, (5,7-dihydroxy-4′-methoxyflavone), a natural flavone, selectively inhibits human atrial repolarization potassium currents and prevents atrial fibrillation. Acacetin is an orally effective atrium-selective agent (Li et al., 2008) (Figure 11).

![Figure 11. Biological activities of flavones](image.png)

**Anti-platelets/Anti-thrombotic activity**

Platelet aggregation is an important pathogenic factor in the development of atherosclerosis and associated thrombosis in humans. Thromboxane B2
formation is a major factor for platelets aggregation. Different flavone and isoflavone derivatives have been found to exhibit anti-platelet and vasorelaxing properties (Ko et al., 2004; Teng et al., 1991). The anticoagulant effects are increased with increasing the number of sulfate groups in the compound. Decasulfated compound was more potent than all other compounds which increase clotting time, clot formation time, and delay the progress of the clot. The possibility of a dual anticoagulant and anti-platelet activities of compounds could enhance their potential as antithrombotic agents (Correia-da-Silva et al., 2011). There is a report showing that oxime- and methyloxime-containing isoflavone-7-y1 derivatives having good anti-platelet activity with respect to flavones (Wang et al., 2005).

**Anti-atherogenic activity**

Flavones inhibit monocyte adhesion to a stimulated endothelium by blocking the induction of cell adhesion molecules on endothelial cells. They have also been found to block TNF-alpha through action on NF-κB transcriptional activation, which induce cell adhesion molecule proteins in human endothelial cells. Apigenin exhibited a reversible effect on cell adhesion molecule expression, and inhibited its upregulation at the transcriptional level (Gerritsen et al., 1995). Luteolin has been reported to interfere with lipopolysaccharide-triggered Akt (PKB) phosphorylation and NF-κB activation (Xagorari et al., 2001; Chen et al., 2004).

Activated endothelium cells and macrophages produce nitric oxide through the activity of nitric oxide synthase (NOS) that is important in maintaining the dilation of blood vessels (Huk et al., 1998). Higher levels of nitric oxide react with
free radicals, producing highly damaging peroxynitrite, which oxidizes LDL, resulting in irreversible oxidative damage of cell membranes. Flavones are well known antioxidants and free radical scavengers. Thus, the flavones scavenge free radicals and making them less available for reacting with nitric oxide, resulting in reduced damage. Nitric oxide itself can be scavenged by flavones (Van Acker et al., 1995). The flavones like kaempferol and apigenin act as the most potent inhibitors of NOS-2 induction via inhibiting NOS-2 gene transcription at micro molar concentrations (García-Mediavilla et al., 2007).

Inflammation plays a central role in the development of atherosclerotic disease. Cyclo-oxygenase-2 (COX-2) enzyme is involved in the formation of inflammatory lesions by catalyzing the conversion of arachidonic acid to prostaglandins. Chrysin and wogonin down-regulates the expression of key pro-inflammatory enzymes like inducible nitric oxide synthase (iNOS) and COX-2. Moreover, flavones with 3’,4’-dichloro substituent’s on the B ring exhibit strong COX-2 inhibitory activities irrespective of the substitution pattern on ring A.

**Anti- hyperlipidaemic activity**

Lipids including cholesterol, cholesteryl esters, phospholipids and triglycerides lead to the pathophysiology of many metabolic diseases like diabetes mellitus, dyslipidemia, CVD, cancer, etc. Hypercholesterolemia is a major risk factor for coronary heart disease. So, the maintenance of lipid levels is important in the treatment of CVD and cerebrovascular diseases (Ntambi and Miyazaki, 2003). The lipids are catabolized through the activation of nuclear receptors like peroxisome proliferator-activated receptors (PPARs) in adipocytes. It has been
reported that polymethoxyflavones significantly increase or activate PPAR-\(\alpha\) and PPAR-\(\gamma\), that further reduced TGs content in the liver and heart. It also regulates adipocytokines by significantly suppressing tumor necrosis factor- \(\alpha\) (TNF-\(\alpha\)), INF-g (interferon-g), IL-1b (interleukin-1b) and IL-6 (interleukin-6) expression (Li et al., 2006). The flavone derivative CM108 has also been reported for lipid regulation. It was observed that this compound increases the HDL level and reduces triglycerides, cholesterol, LDL in serum and liver (Guo et al., 2006). The hybrid congener’s 6- and 7-hydroxy flavones with aminopropanol have been synthesized and evaluated for anti-dyslipidemic activities. Bulky lipophilic substitution like tert-butyl- on ring-B of flavones, the oxygen atoms at the 3’,5’-positions in the B ring seem necessary for antidyslipidemic activities (Pratap et al., 2009).

**Vasorelaxant activity**

Series of flavones like 3-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone and chrysin have been evaluated for ex-vivo and *in-vitro* vasorelaxant effect, therefore, can be the drugs of interest as novel antihypertensive agents. All the flavone derivatives possess endothelium-dependent vasorelaxant effect, with an increased production of nitric oxide and prostacyclin PGI2 in a concentration-dependent manner (Torres-Piedra et al., 2011).

Dong et al (2009), designed and synthesized several flavones derivatives for vasorelaxant activity. Hydroxyl group at C-5 and C-7 positions in the ring-A, C-4 carbonyl group, C2=C3 double bond and hydroxyl groups at different positions in ring-B are important features for the vasorelaxant activity, while the
presence of C-glycosyl group at C-8, hydroxyl group at the C-3 position, greatly reduce relaxation effect. On the other hand, bulky substituents at ortho- and meta-positions on ring-B of flavone derivatives decrease the potency of vasorelaxant activities. 5, 7-dihydroxy-3’-bromo-flavone exhibits the highest vasodilator activity (Chen et al., 2004).

**Diosmin**

Diosmin (3’, 5, 7-trihydroxy-4’-methoxyflavone 7-rutinoside) (Figure-12) is an unsaturated flavone that can be found mainly in Hyssop and Rosemary (Del Baño et al., 2004; Camarda et al., 2007). It has been receiving much attention because of its wide array of biological properties.

**Pharmacological actions of diosmin**

Diosmin is considered as a vascular-protecting agent in the treatment of hemorrhoids, lymphedema, varicose veins and different types of cancer (Camarda et al., 2007; Le Marchand et al., 2000).

![Figure 12. Structure of diosmin (3’, 5, 7-trihydroxy-4’-methoxyflavone 7-rutinoside)](image)
**Physicochemical properties of diosmin**

Chemical abstract service No : 520-27-4  
Chemical name : Diosmin  
Synonyms : Daflon;  
Molecular formula : $\text{C}_{28}\text{H}_{32}\text{O}_{15}$  
Formula weight : 608.54  
Melting point : 275-277°C  
Storage temperature : 2-8°C  
Solubility : 1 M NaOH: 10 mg/mL, normal saline  
Form : Powder  
Color : grayish-yellow or light yellow  
Water solubility : $<0.1$ g/100 ml at 21 ºC  
Sensitivity : Air and light sensitive  
Stability : Stable under normal condition, hygroscopic

**Anti-apoptotic effect**

Liu et al. (2014) described the effect of diosmin against cerebral ischemia. Diosmin provided defence against cerebral ischemia/reperfusion injury in mice and exerted effect of anti-apoptosis in tMCAO mice. The ability of diosmin to activate JAK2/STAT3 signal pathway was also reported.

**Chemopreventive effect**

A study by Tahir et al. (2013) revealed that diosmin being a dietary supplement, could be used as chemopreventive agent to prevent hepatocarcinogenesis. Diosmin exerts a chemopreventive effect against experimentally induced *in vivo*...
hepatocarcinogenesis in rats. The dose-responsive chemopreventive properties of diosmin have been reflected in its ability to abrogate the development of preneoplastic hepatic nodule formation. The inhibition of cell proliferation and downregulation of inflammatory markers may be the part of underlying mechanisms related to the liver tumor inhibition by diosmin.

**Anti-tumor effect**

A study by Tran Duc Dung et al., (2012) indicates that diosmin significantly promotes HA22T apoptosis and reduces tumour size in xenograft nude mice via PP2A in a dose-dependent manner.

**Anti-hyperglycemic effect**

According to Pari and Srinivasan (2010), the administration of diosmin resulted in a significant restoration of plasma glucose, insulin, glycosylated hemoglobin and the activities of carbohydrate metabolic enzymes in streptozotocin-nicotinamide-induced diabetic rats, by its antidiabetic effect.

**Anti-hypertensive effect**

Diosmin possesses an antihypertensive effect which is evidenced by lowered blood pressure, lipid peroxides, improved nitric oxide availability and antioxidant status (Silambarasan and Raja, 2012).

**Anti-hyperlipidemic activity**

Srinivasan and Pari (2013) reported that the administration of diosmin to diabetic rats reduced the plasma and tissue levels of lipids and lipid metabolizing
enzymes to near normal levels. The treatment of diosmin showed beneficial effects in the prevention and controlling of dyslipidemia in diabetic rats.

Literature survey revealed there are no scientific reports available on the effects of diosmin on myocardial infarction.