CHAPTER 1

INTRODUCTION

1. INTRODUCTION

In connection with the project entitled, it is necessary to describe the normal functioning of the heart and thereby its pathophysiological conditions. The cardiovascular system refers to the heart, blood vessels and the blood. The heart is the muscular organ of the circulatory system that constantly pumps blood with nutrients throughout the body. The heart is composed of cardiac muscle tissue that is very strong and able to contract and relax rhythmically throughout lifetime. The upper chamber on each side of the heart, which is called an atrium, receives and collects the blood coming to the heart. The atrium then delivers blood to the powerful lower chamber, called a ventricle, which pumps blood away from the heart through powerful, rhythmic contractions. Electrical impulse from heart muscle (the myocardium) contracts the heart. This electrical signal begins in the sinoatrial (SA) node, located at the top of the right atrium. The SA node is sometimes called the heart's "natural pacemaker." An electrical impulse from this natural pacemaker travels through the muscle fibers of the atria and ventricles, causing them to contract.

The human heart is actually two pumps in one. The right side receives oxygen-deficient blood from the various regions of the body and delivers it to the lungs. In the lungs, oxygen is absorbed in the blood. The left side of the heart receives the oxygen-rich blood from the lungs and delivers it to the rest of the body. The essential function of the heart is to pump blood to various parts of the body. The mammalian heart has four chambers: right and left atria and right and left ventricles.

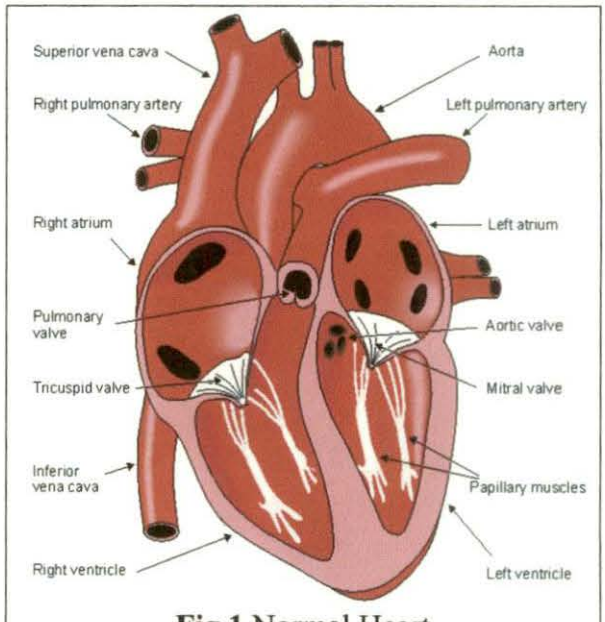


Fig.1. Normal Heart

The two atria act as collecting reservoirs for blood returning to the heart while the two ventricles act as pumps to eject the blood to the body. The heart comes complete with valves to prevent the back flow of blood as in any pumping system. Deoxygenated blood returns to the heart via the major veins (superior and inferior vena cava), enters the right atrium, passes into the right ventricle and from there is ejected to the pulmonary artery on the way to the lungs. Oxygenated blood returning from the lungs enters the left atrium

via the pulmonary veins, passes into the left ventricle, and is then ejected to the aorta which then distributes it to the rest of the body through various arteries and the heart muscle through coronary arteries. Also branching off the aorta as it leaves the heart is a pair of coronary arteries, these arteries supply blood to the heart muscle and are considered as a part of the systemic circulation. After passing through capillaries in the heart, blood in the coronary circuit returns to the right side of the heart through veins that empty directly into the right atrium. Heart attacks are caused by clots in coronary arteries, depriving the heart muscle of oxygen.

1.1. ISCHEMIC HEART DISEASE (MYOCARDIAL ISCHEMIA)

Coronary artery disease is a condition in which fatty deposits (atheroma) accumulate in the cell lining of the coronary arteries. These fatty deposits build up gradually and irregularly in the large branches of the two main coronary arteries which supplies blood to the heart muscle. This process is called atherosclerosis which leads to narrowing or hardening of the coronary arteries and arresting blood supply to the heart muscles. This results in ischemia in heart muscles which can damage them. An obstruction of coronary arteries develops gradually from the accumulation of fatty, fibrous plaques that narrow the lumen of coronary artery, reduce the blood flow and lead to infarction. The complete occlusion of the coronary arteries leads to myocardial infarction and ultimately death (**Fig .2.**)

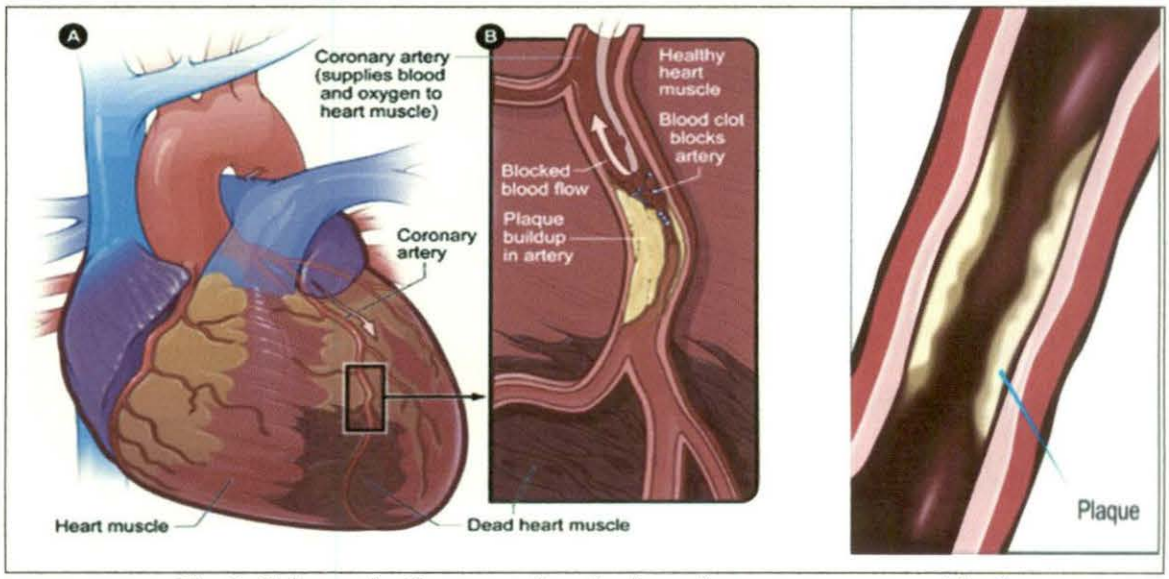


Fig.2. Schematic diagram of occlusion of coronary artery with plaque

An infarct is an area of tissue that has died because of lack of oxygenated blood. Myocardium is affected when a branch of a coronary is occluded. The extent of myocardial damage depends upon the size of the blood vessel and site of the infarct. The damage is permanent because cardiac muscle cannot regenerate and the dead tissue is replaced with non functional fibrous tissue. Speedy restoration of blood flow through the blocked artery using thrombolytic drugs can greatly reduce the extent of permanent damage and improve the prognosis but treatment must be started within a few hours of the infarction occurring. The myocardial infarction is usually accompanied by severe crushing chest pain which radiates to the left arm, jaw and neck and continues even when the individual is at rest.

Cardiovascular disease is a general name for a wide variety of diseases, disorders and conditions that affect the heart and the blood vessels as well. It includes angina, myocardial infarction, atherosclerosis, heart failure, ischemic heart disease and cardiac arrhythmias. The other forms of cardiovascular disease include congenital heart defects, cardiomyopathy, coronary artery disease, heart valve disorders, myocarditis, pericarditis and infections of the heart. Symptoms of cardiovascular disease vary depending on the specific type of cardiovascular disease. A classic symptom of cardiovascular disease is chest pain. It has the characteristic distribution in the chest, left arm, neck, and brought to the shoulder blade.

Risk factors for developing cardiovascular disease are hypertension, diabetes, high cholesterol (hyperlipidemia), obesity, and a sedentary lifestyle. Other risk factors include drinking excessive amounts of alcohol, having a lot of long term stress, smoking and having a family history of a heart attack at an early age.

Ischemic heart disease is the leading cause of death worldwide, and 3.8 million men and 3.4 million women die of the disease each year. After an acute myocardial infarction, early and successful myocardial reperfusion with the use of thrombolytic therapy (eg.streptokinase) or primary percutaneous coronary intervention (PCI) is the most effective strategy for reducing the size of a myocardial infarct and improving the clinical outcome.

1.2. MYOCARDIAL ISCHEMIC REPERFUSION INJURY

Advent of early coronary recanalization for limiting morbidity and mortality due to myocardial infarction has brought forward a menace in the form of reperfusion injury.

Ischemia is the condition in which the organ is derived from blood flow followed by inadequate oxygen and nutrient supply. Although restoration of blood flow to an ischemic organ is essential to prevent irreversible cellular injury, reperfusion itself may augment tissue injury in excess of that produced by ischemia alone. Reperfusion of the previously-ischemic myocardium is often followed by the detrimental changes in coronary arteries and myocardial tissues, which ultimately results in cardiac dysfunction, known as ischemia/reperfusion (I/R) injury.

The process of restoring blood flow to the ischemic myocardium, however, can induce injury. This phenomenon, termed myocardial reperfusion injury (Yello *et al.*, 2007), can paradoxically reduce the beneficial effects of myocardial reperfusion. Healing of myocardial infarcts depends on an inflammatory cascade that ultimately results in clearance of dead cells and matrix debris and formation of a scar. Myocardial reperfusion injury was first postulated in 1960 by Jennings et al. in their description of the histologic features of reperfused ischemic canine myocardium. The observed features are cell swelling, contracture of myofibrils, disruption of the sarcolemma, and the appearance of intramitochondrial calcium phosphate particles. The injury to the heart during myocardial reperfusion causes four types of cardiac dysfunction. The first type is myocardial stunning, a term denoting the "mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage and despite restoration of normal or near-normal coronary flow (Barunwald *et al.*, 1982). The myocardium usually recovers from this reversible form of injury after several days or weeks. The second type of cardiac dysfunction, the no-reflow phenomenon, was originally defined as the "inability to reperfuse a previously ischemic region (Krug *et al.*, 1966) it refers to the impedance of micro vascular blood flow encountered during opening of the infarct-related coronary artery. The third type of cardiac dysfunction, reperfusion arrhythmias, is potentially harmful, but effective treatments are available (Mannings *et al.*, 1984). The last type is lethal reperfusion injury (Kloner *et al.*, 1993). The mediators which are responsible for this type are as follows.

1.2.1. Potential mediators of lethal reperfusion injury

1.2.1.1. Oxygen paradox

Experimental studies have established that the reperfusion of ischemic myocardium generates oxidative stress which itself can mediate myocardial injury. Oxidative stress is part of the oxygen paradox in which the reoxygenation of ischemic myocardium generates a degree of myocardial injury that greatly exceeds the injury induced by ischemia alone; the role of oxidative stress in lethal reperfusion injury is clouded by the inconclusive results of animal and clinical studies of cardio protection by antioxidant reperfusion therapy.

Oxidative stress during myocardial reperfusion also reduces the bioavailability of the intracellular signaling molecule, nitric oxide, thereby removing its cardioprotective effects. These effects include the inhibition of neutrophil accumulation, inactivation of superoxide radicals and improvement of coronary blood flow. The Nitric oxide reperfusion therapy to increase nitric oxide levels can reduce the size of a myocardial infarct in animals but clinical studies of the anti anginal nitric oxide donor nicorandil have reported benefit only in terms of improved myocardial reperfusion; results in terms of clinical outcomes after an acute myocardial infarction are mixed (Ono *et al.*, 2004; Ishii *et al.*, 2005).

1.2.1.2. Calcium paradox

At the time of myocardial reperfusion, there is an abrupt increase in intracellular Ca^{2+} which is secondary to sarcolemmal-membrane damage and oxidative stress induced dysfunction of the sarcoplasmic reticulum. These two forms of injury overwhelm the normal mechanisms that regulate Ca^{2+} in the cardiomyocyte, this phenomenon is termed the calcium paradox. The result is intracellular and mitochondrial Ca^{2+} overload and this excess of Ca^{2+} induces cardiomyocyte death by causing hypercontracture of the heart cells and mitochondrial PTP opening. Attenuating intracellular Ca^{2+} overload with pharmacologic antagonists of the sarcolemmal Ca^{2+} ion channel, the mitochondrial Ca^{2+} uniporter or the sodium hydrogen ion exchanger decreases myocardial infarct size by up to 50% in experimental studies. However, the results of the corresponding clinical studies have been negative that inhibition of sodium-hydrogen ion exchange at the time

of PCI does not protect the myocardium. During an acute myocardial infarction is consistent with the results of experimental studies in which the beneficial effects of inhibiting sodium-hydrogen ion exchange were shown to occur during myocardial ischemia and not reperfusion, the new class of agents that reduce intra cellular Ca^2+ loading by inhibiting the sodium hydrogen exchanger and promoting Ca^2+ uptake by the sarcoplasmic reticulum has also not influenced infarct size when given during reperfusion.

1.2.1.3. Inflammation

After an acute myocardial infarction, the release of chemo attractants draws neutrophils into the infarct zone during the first 6 hours of myocardial reperfusion and during the next 24 hours they migrate into the myocardial tissue. This process is facilitated by cell-adhesion molecules. These neutrophils cause vascular plugging and release degradative enzymes and reactive oxygen species. Experimental studies have shown reductions in infarct size of up to 50% with several interventions aimed at neutrophil during myocardial reperfusion. These interventions include leukocyte depleted blood; antibodies against the cell-adhesion molecules P-selectin 64, CD11 and CD18, 65 and the intercellular adhesion molecule 166; and pharmacologic inhibitors of complement activation. However, the corresponding clinical studies have not shown any meaningful cardio protective effect of such interventions.

After inconclusive experimental studies, clinical studies of the anti-inflammatory agent adenosine as an adjunct to PCI have shown an 11% reduction in the size of myocardial infarcts, but benefits in terms of clinical outcomes were limited to patients presenting within 3 hours after the onset of symptoms (**Fig.3**)

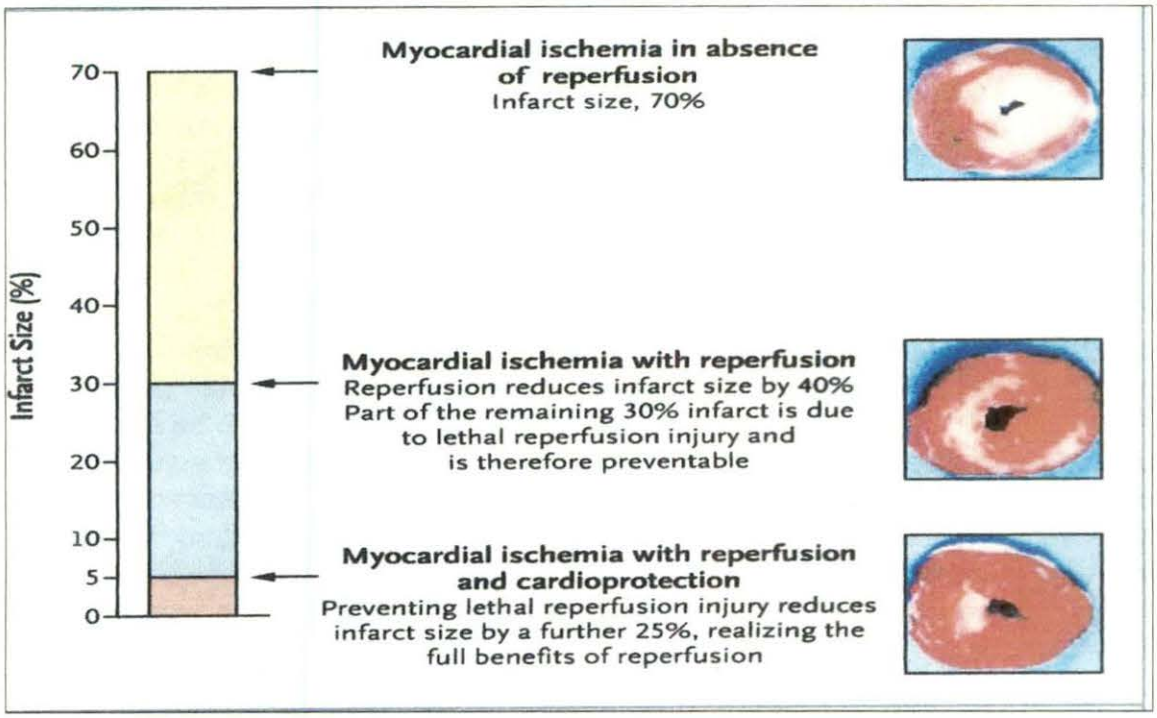


Fig.3. Contribution of lethal reperfusion injury to final myocardial infarct size.

1.2.2. Cardioprotective strategies for preventing lethal myocardial reperfusion injury

Targeting individual mediators of lethal reperfusion injury has produced discrepant findings in studies in animals and clinical studies that use this strategy have not been successful. A more effective approach may be to target more than one mediator at a time. The recently described interventional strategy of ischemic post conditioning which by its nature targets several mediators of lethal reperfusion injury and it has been shown to reduce myocardial injury in patients with acute myocardial infarction who are undergoing PCI (Staat *et al.*, 2005). These findings along with a number of preclinical studies have not only re-ignited interest in the myocardial reperfusion phase as a target for cardio protection, but they also have provided confirmatory evidence of the existence of lethal re perfusion injury in humans Further more, the RISK pathway (Hausenloy *et al.*, 2006), and the mitochondrial PTP are emerging as new targets for preventing lethal reperfusion injury.

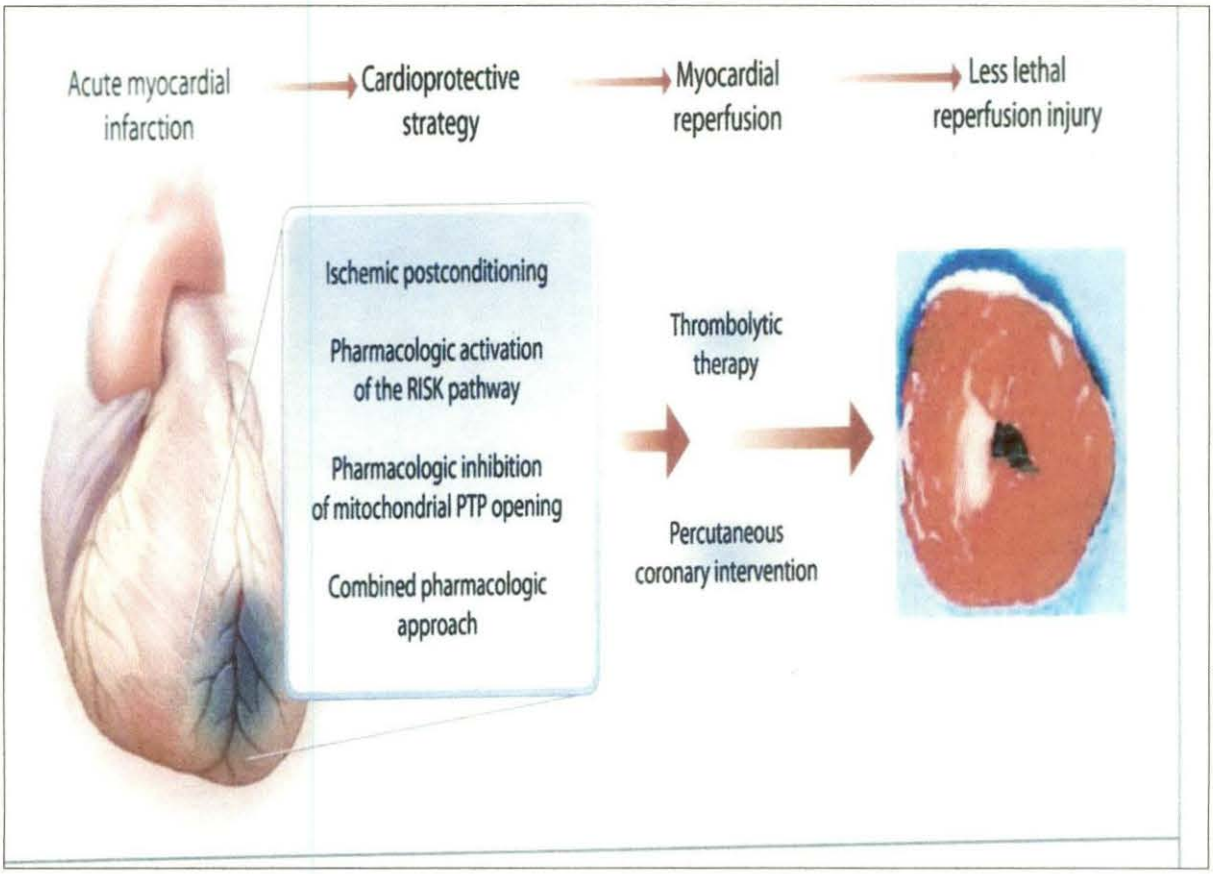


Fig.4. New cardioprotective strategies for reducing lethal reperfusion injury

including thrombolysis and primary coronary angioplasty improve survival after myocardial infarction (MI) (Ryan *et al.*, 1996). Unfortunately, these reperfusion therapies are rarely carried out before considerable myocardial injury has occurred. Moreover, reperfusion after prolonged ischemia also produces a paradoxical myocardial injury (ischemia-reperfusion injury) (Ambrosio *et al.*, 1991; Forman *et al.*, 1990) potentially limiting the efficacy of reperfusion therapies. This has provided impetus for identifying therapies that protect against ischemia-reperfusion injury. Acute interventions including ischemic preconditioning (Jenkins *et al.*, 1995; Parratt, 1995) and infusion of adenosinergic agents (Downey *et al.*, 1993; Forman *et al.*, 1993) reduce ischemia-reperfusion injury in animal models and human myocardium. Unfortunately, the timing of an MI cannot be predicted clinically thus we need to develop therapies that produce sustained protection against ischemia-reperfusion injury in vulnerable patients.

Several lines of evidence suggest that nucleoside transport inhibitors, which increase extracellular adenosine levels by inhibiting uptake into myocytes and endothelial cells might offer sustained protection against ischemia-reperfusion injury (**Fig.4**). For example, we recently found that chronic exposure to ethanol, an adenosine uptake inhibitor, reduces ischemia-reperfusion injury in guinea pig hearts, requiring adenosine A_1 receptor signaling at the time of ischemia to effect cardioprotection (Miyamae *et al.*, 1997). Other nucleoside transport inhibitors including dipyridamole (Amrani et al., 1992; Auchampach and Gross, 1993; Gokgoz *et al.*, 1992) also reduce ischemia-reperfusion injury when infused immediately before experimental MI. However, still it is unknown whether these agents offer sustained protection against ischemia-reperfusion injury when given chronically.

1.3. TREATMENT FOR ISCHEMIC HEART DISEASE

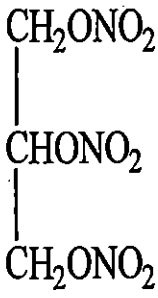
There are currently three types of medicines used to treat stable angina are as follows.

- Nitrates
- Beta blockers
- Calcium channel blockers
- Drugs from natural origin sources/ Herbal remedies

1.3.1. Nitrates

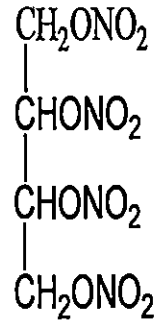
Nitrates improve the blood flow by relaxing and dilating veins and arteries including the coronary arteries. Some of the nitrate preparations available in the market are as follows.

- Short acting: Glyceryl trinitrate (GTN, Nitroglycerine)
- Long acting: Isosorbide dinitrate, Isosorbide mononitrate and Erythirtyl Tetranitrate



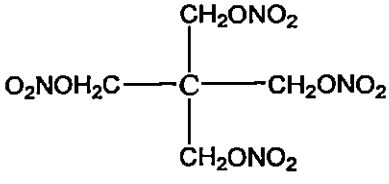
Nitroglycerin

Drugs of choice for Unstable Angina



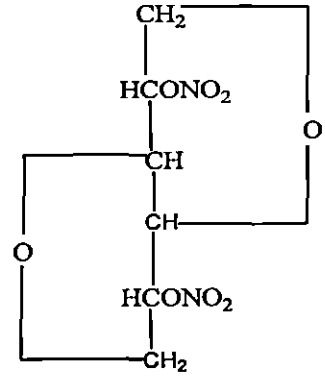
Erythrityl Tetranitrate

Drug used in the prophylaxis of Angina pectoris,cyanide poisoning &CHF



Pentaerythritol Tetranitrate

Drug used in the prophylaxis of Angina pectoris



Isosorbide Dinitrate

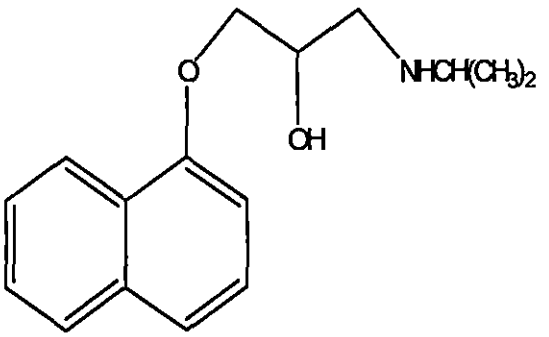
Drug of choice for Angina pectoris only for prophylactic use

1.3.2. Beta blockers

Beta blockers reduce the heart rate, blood pressure and the force of contractions, thereby decreasing the amount of oxygen which the heart requires to pump blood. Along with nitrates, beta blockers are usually the first choice for the treatment of stable angina. There are different types of beta blockers and, although all are equally effective in the

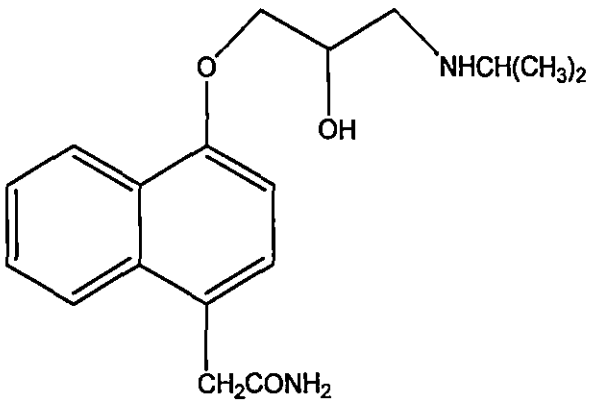
treatment of angina.Nonselective beta blockers (propranolol) block all types of beta receptors throughout the body and are therefore more likely to cause side effects.

Cardio selective beta blockers (atenolol and metoprolol) selectively block the beta receptors found in the heart. Some beta blockers (acebutolol and pindolol) are less likely to depress cardiac function or cause a slow resting heart rate and may be a better choice for people who have specific cardiac conditions or more sensitive to the effects of beta blockers. Some beta blockers (labetalol or carvedilol) also block alpha receptors, which are another type of receptor found in the blood vessels. These medications have the added benefit of dilating blood vessels.



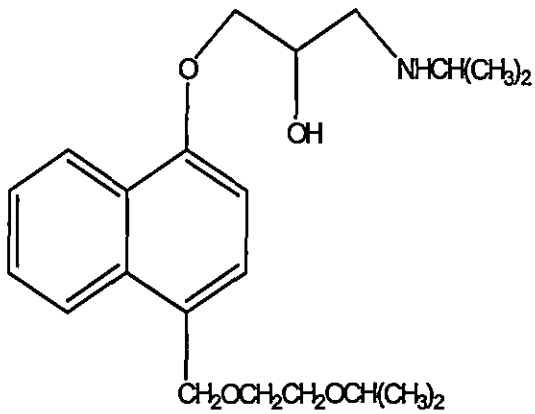
Propranolol

Drug used for the treatment of Hypertension, Angina, Myocardial Infarction and Arrhythmia

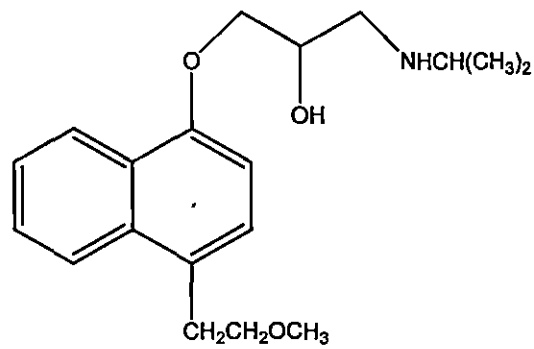


Atenolol

Drug used in the treatment of Angina and Hypertension



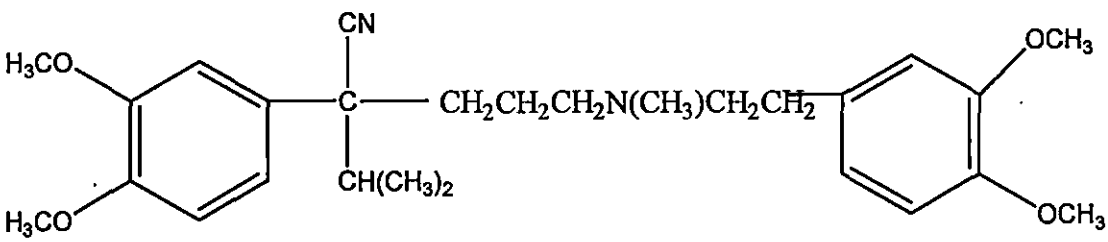
Bisoprolol



Metoprolol

It is used in Congestive Heart Failure & Hypertension

Drug of choice for Angina, Cardiac Arrhythmia & CHF



Verapamil

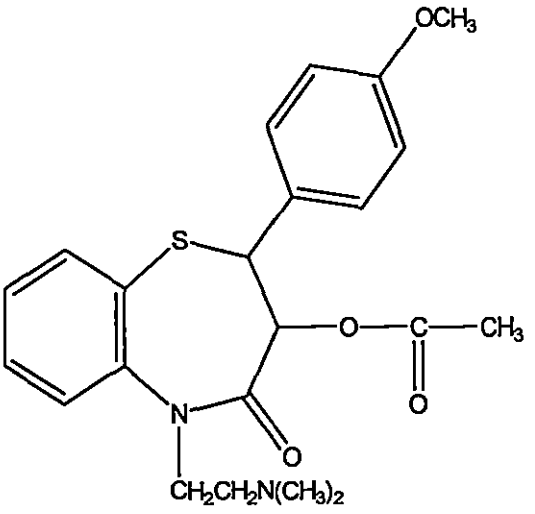
Drugs used for the treatment of Hypertension, Angina & Arrhythmia

1.3.3. Calcium channel blockers

Calcium channel blockers dilate arteries and lower blood pressure which decreases the force of the heart's contractions. They also dilate veins, reducing the amount of blood returning to the heart, which reduces the workload of the heart. It includes dihydopyrimidine derivatives, diltiazem and verapamil. The dihydropyridine includes nifedipine, nicardipine, Felodipine, Amlodipine, Nitrendipine, Nimodipine, Lacidipine, Lercanidipine and Benidipine etc.

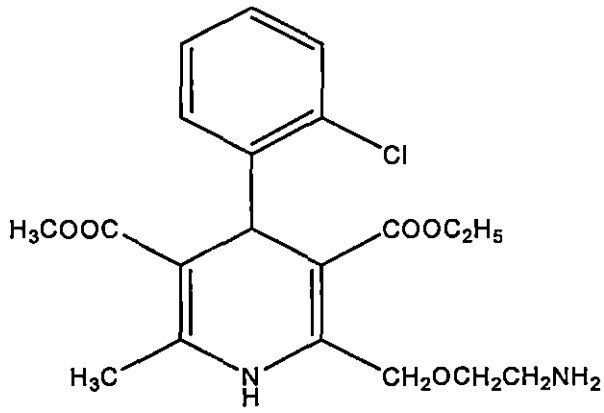
Verapamil slows the heart's conduction of electrical impulses, decreases the force of the heart's contractions, and dilates blood vessels. Although it is less effective than beta blockers for slowing the heart rate, it is a safe and effective alternative.

Diltiazem dilates blood vessels (especially coronary arteries), decreases the heart's force of contraction, and slows the heart's conduction of electrical impulses. It is available in sustained release form that is taken once per day. Anti-cholesterol drugs called statins, which help to lower blood cholesterol may also be used to reduce greater plaque buildup in the coronary arteries.



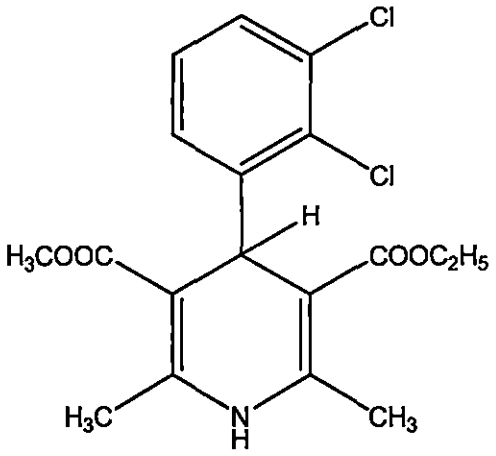
Diltiazem

It is a potent coronary vasodilator

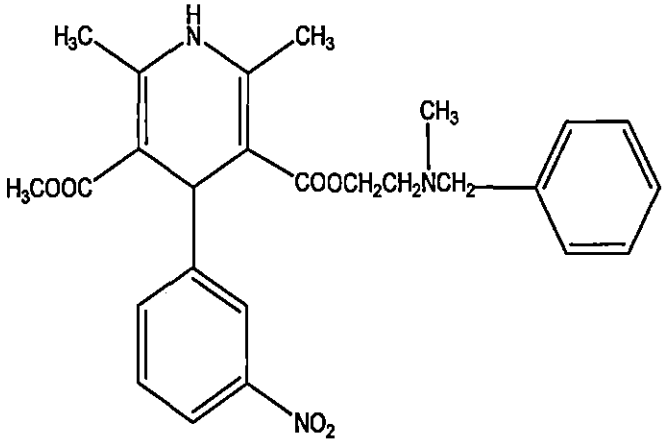


Amlodipine

Drug of choice for Angina pectoris



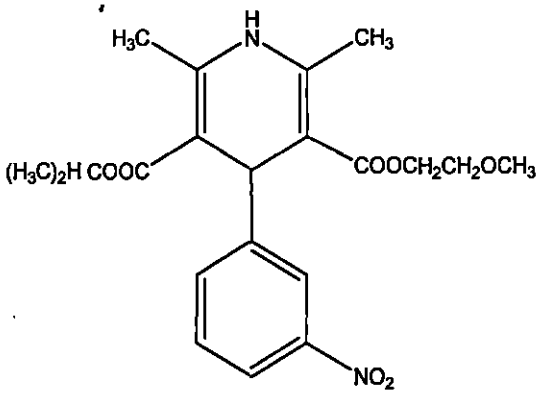
Felodopine



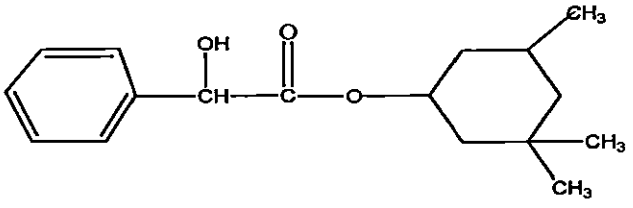
Nicardipine

Used in the treatment of Hypertension

Drug of choice for Myocardial infarction



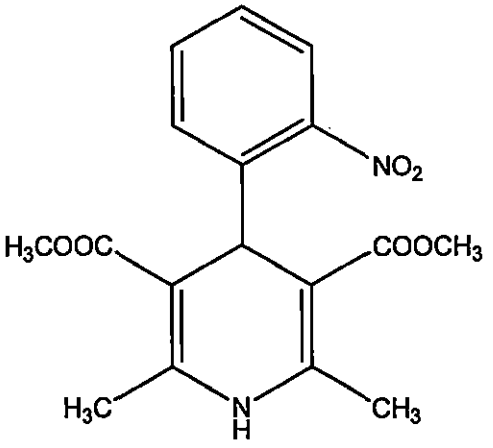
Nimodipine



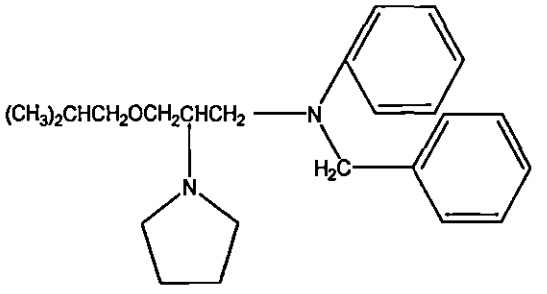
Cyclandelate

Drug of choice for myocardial infarction

Drug used for the treatment of Thrombophlebitis & Raynaud's Disease



Nifedipine

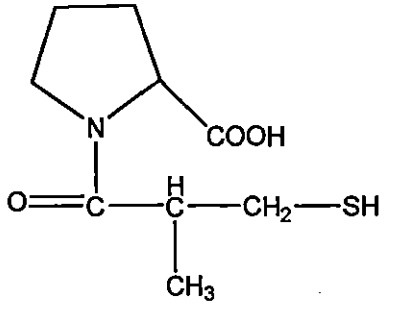


Bepridil

Used in the treatment of Angina, Hypertension & Arrhythmia

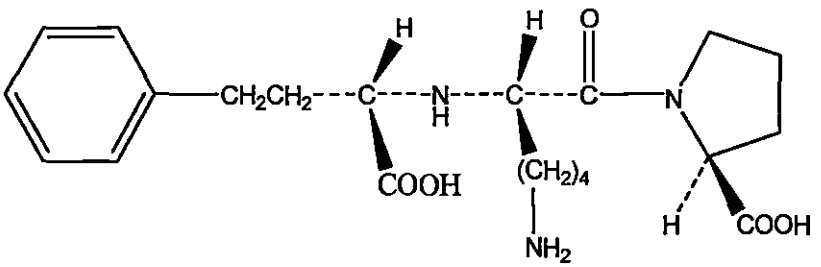
Drug of choice for Angina & Arrhythmia

Angiotensin converting enzyme inhibitors includes the following drugs captopril, enalpril, lisinopril, benazepril, ramipril, fosinopril, trandolapril, imidapril and perindopril. ACE inhibitors prevent the conversion of angiotensin-I to angiotensin- II (active octapeptide).



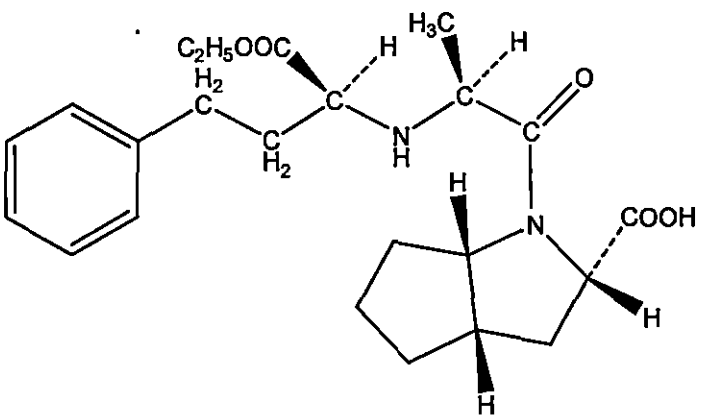
Captopril

It is effective in renal & malignant hypertension



Lisinopril

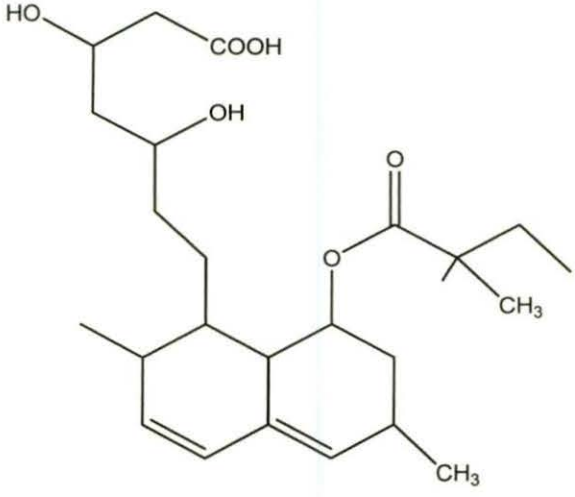
It is used in CHF & Heart Failure



Ramipril

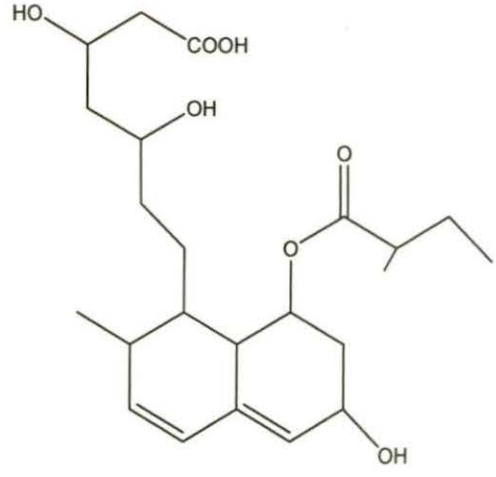
It is used in CHF & Heart failure

Lovastatin are the drugs called HMG CoA reductase inhibitors. They inhibit the synthesis of cholesterol which is responsible for the formation of plaque in coronary arteries.



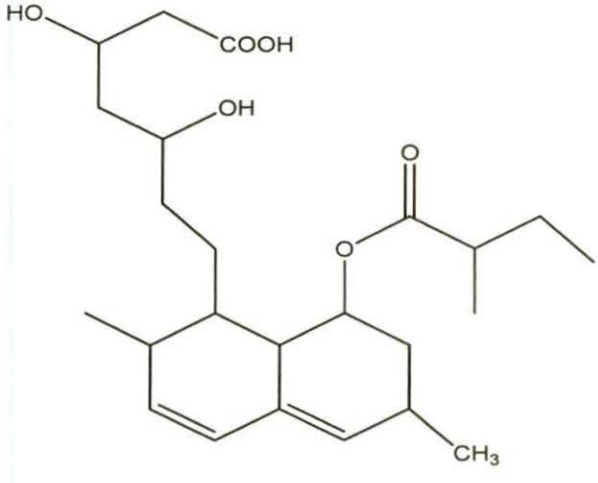
Simvastatin

It is used in diabetes mellitus for its LDL Lowering effects



Pravastatin

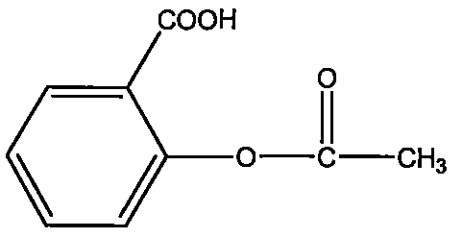
It is used in treatment of diabetes mellitus for its LDL Lowering effects



Lovastatin

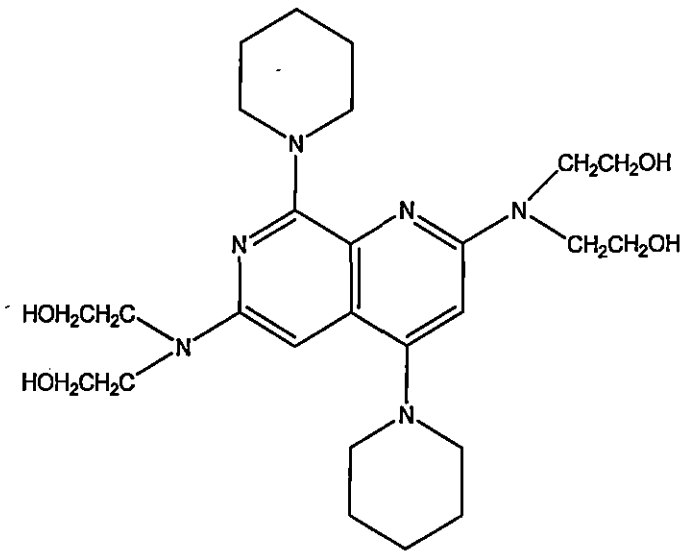
It is used in treatment of diabetes mellitus for its LDL Lowering effects

Anti-platelet drugs- such as aspirin are recommended for patients with coronary artery disease. Aspirin binds irreversibly to platelets and prevents them from clumping on blood vessel walls- thus preventing platelets from forming a clot on the fatty plaques which could block an artery and result in heart attack.



Aspirin

It is used as fibrinolytic agents



Dipyridamole

It is used as antiplatelet agent

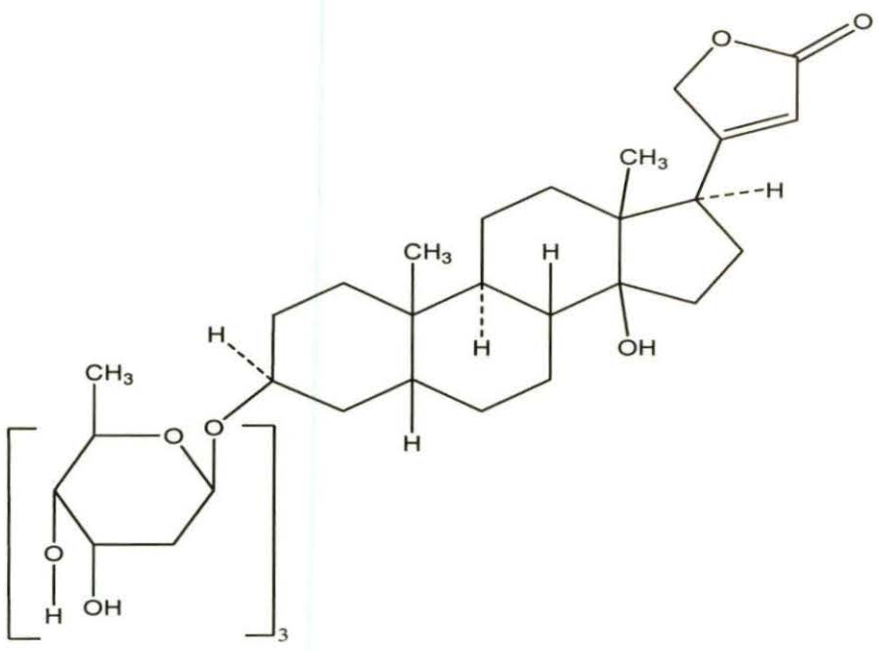
1.3.4. Herbal remedies

Numerous literatures related to cardioprotective activity of phyto molecules from herbal sources have shown that there is a vast array of phytoconstituents having cardio protective efficacy. Resveratol is one of the potent molecules reported for their cardioprotective activity. The cardioprotective activity of resveratol is associated with the inhibition of platelet aggregation and LDL oxidation and the promotion of artery vasorelaxation. The natural sources such as sesame oil, abana, terminalia arjuna and allium sativum etc are being used to treat cardiovascular disease ((Szmitko *et al.*, 2005; Mukherjee *et al.*, 2003; Kaneez *et al.*, 2007).

The history of herbal medicines is as old as human civilization. The documents many of which are of great antiquity revealed that plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era. A large portion of the Indian population even today depends on the Indian system of medicine -Ayurveda "An ancient science of life" a discipline under Atharvaveda since the Vedic age dating nearly 2000 B.C.The Hindu materia medica like "Charak Samhita" and Sushrata Samhita and the later the Egyptian document "Ebers Papyrus" are the earliest written treaties to record the disease along with its symptoms and a number of possible remedies (Holcomb, 1963)

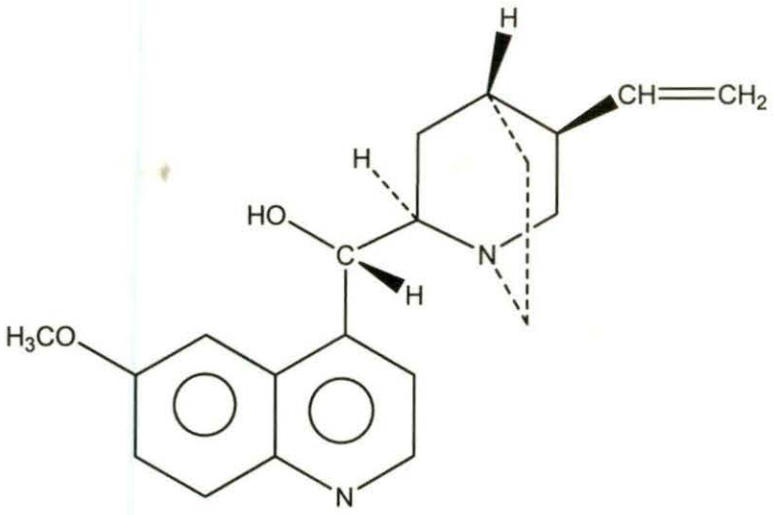
Some examples of common herbal /ayurvedic cardiovascular drugs are mentioned below.

Digoxin,a steroid glycoside from Digitalis lanata used in the treatment of congestive heart failure, it also finds use in the treatment of arrthymia.



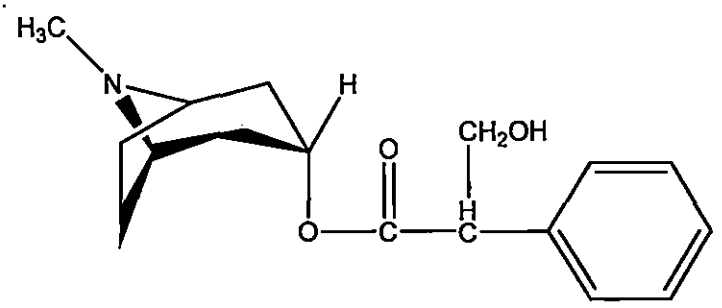
Digoxin

Quinidine, a stereo isomer of the antimalarial quinine, found in cinchona bark, is acting as an adequate antiarrythimic agent but has recently been replaced by pace maker and newer drugs.



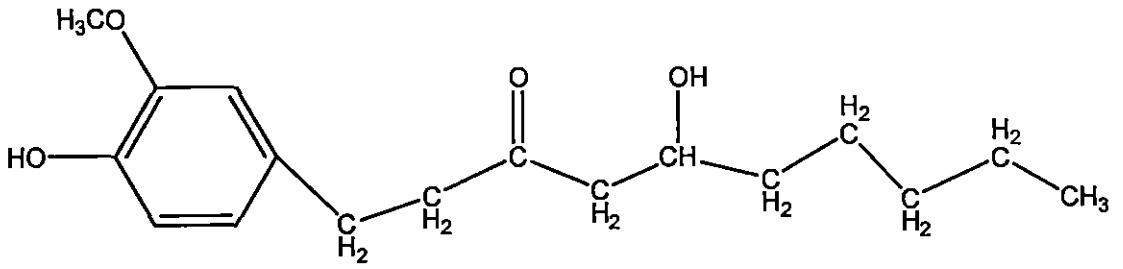
Quinidine

Slow heart beat (bradycardia) is treated with Atropine, a metabolite of Solanaceus plant Atropa belladonna.



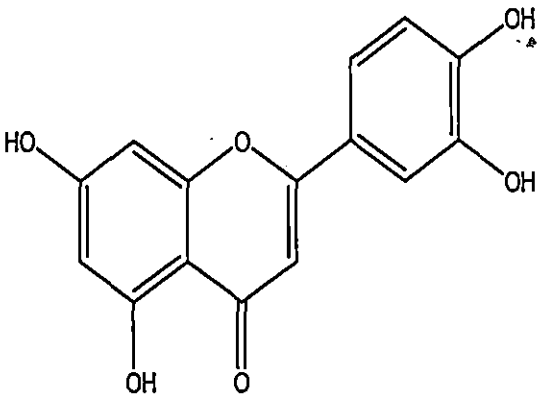
Atropine

The relatively simple phenol-gingerol from the rhizomes of Zingeber officinale (Zingiberaceae) appears to have new cardiotonic action.

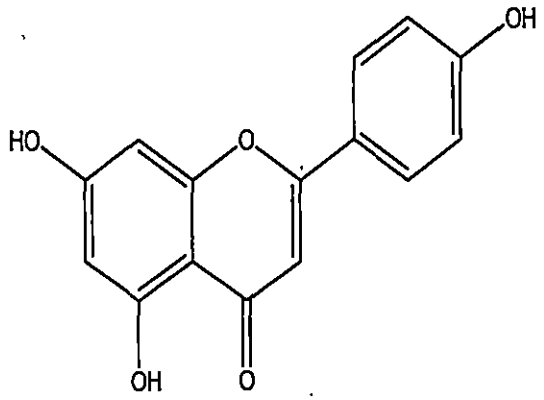


Gingerol

There are many Ayurvedic drugs such as as Arjuna (*Terminalia arjuna*) used as cardio tonic. Many drugs containing flavones are also showing cardio tonic activity.

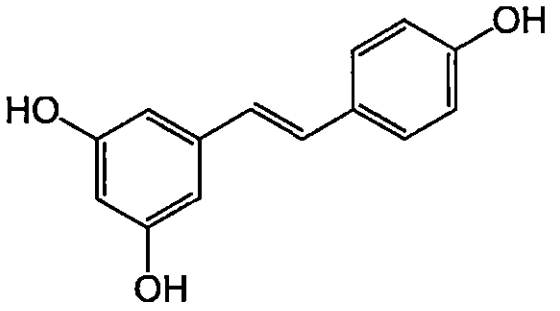


Flavone



Flavonol

This 'french paradox' has been assigned in part to consumption of red wine which contains resveratol, thought to be responsible for the cardiovascular benefits(Hung *et al.*, 2000). A vast number of pharmacological investigations have substained the therapeutic potential of Resveratol, a phyllotoxin group of polyphenol found naturally in grapes (King *et al.*, 2006).Resveratrol has been shown to dose-dependently inhibit induced production of PGE2 in human peripheral blood leukocytes (Richard *et al.*, 2005, while significantly decreased levels of rat PGD2 in vivo models (Martin *et al.*, 2004). Further, it has antioxidant activity associated with chemo-preventive and cardioprotective activity (Lin and Sai, 1999).



Resveratrol

It is effective against atherosclerosis & CHF

1.3.5. YOGA AND CARDIOVASCULAR DISEASE

The ancient marvel of yoga is the priceless gift of India to the world. Yoga is beneficial in health as well as disease as it is holistic and has promotive, preventive as well as curative potential. Our body, mind and spirit are intricately interrelated and constantly influence one another. The holistic science of yoga has been designed to have subtle effect on our whole being, body, mind as well as spirit. The all pervasive stress and stress-induced disorders like hypertension and angina are fast growing epidemics and bane of "modern" society. The holistic science of yoga is the best method for prevention as well as management of stress and stress-induced disorders. The psycho physiological responses to yoga are opposite to the stress response. Shavasan, yoganidra, meditation and slow, rhythmic pranayam breathing are very effective in calming the mind and

promoting psychosomatic health. Cardiac patients are sensitive and reactive. Yoga relaxation techniques calm the mind and make one emotionally balanced. Consequently, minor disturbances do not cause emotional upsets and cardiovascular problems. Throughout the world, hypertension is a common condition and many patients are on life-long medication as a way of life. Drugs are expensive and have many adverse side effects. Hence, nondrug management like yoga should be the first choice. If diagnosed early, majority of the cases of essential hypertension can be managed effectively by yoga alone. In more advanced cases, yoga can decrease drug dosage and improve the overall quality of life. Besides being inexpensive, safe and effective, yoga improves overall health and can be combined with allopathic or ayurvedic medication. For best results, yogic lifestyle should be adopted early in life as it has been demonstrated that atherosclerotic plaques in coronary arteries form early in life.

Moreover, in a recent study, it has been demonstrated that the levels of total and LDL cholesterol are higher in pre hypertensive as compared to normotensive subjects (Pavithran *et al.*, 2007). Hence, yogic relaxation and yogic diet should be adopted early in life to prevent progression of the condition and development of hypertension. The effectiveness of yoga in the management of hypertension has been demonstrated from our laboratories (Vijayalakshmi *et al.*, 2004) and also by earlier workers (Datey *et al.*, 1969. Hence, it is recommended that yogic relaxation techniques should be adopted as the first line of treatment for pre hypertension, borderline hypertension and mild hypertension. Yoga has therapeutic potential in other conditions also.

In an interesting study, it was recently demonstrated that yoga relaxation training is beneficial in patients with benign ventricular ectopies (Ravindra *et al.*, 2005). Therapeutic effect of yoga may be due to i) management of stress ii) improvement of cardio respiratory function and overall fitness and iii) modulation of autonomic function. Stress is an important causative factor in cardiovascular diseases like hypertension and angina. In an interesting work from research laboratories, it was demonstrated that subjects trained in yoga can achieve a state of deep psychosomatic relaxation associated with highly significant decrease in oxygen consumption within five minutes of practicing savitri pranayam (a slow, rhythmic and deep breathing) and shavasan. These findings are consistent with the report that yoga training not only produces a significant decrease in

basal anxiety level, but also attenuates the change in anxiety score in stressful situations such as examination (Malathi and Damodaran, 1999). It has also been reported that yoga training helps in development of resistance against stress (Udupa and Singh, 1972). Practice of asans and pranayams results in overall improvement in physical fitness and cardio-respiratory functions. In a study conducted on medical students, it was found that yoga training of 12 weeks duration produces a significant increase in respiratory pressures, breath holding time and handgrip strength. This indicates an improved physical strength and cardio-respiratory function. It was reported that after yoga training, exercise-induced stress to cardiovascular system is less severe (Madanmohan *et al.*, 2004). Yoga training promotes emotional and physiological balance. In an interesting study, it was found that a brief (15 min) yoga based relaxation training normalizes the function of autonomic nervous system by deviating both sympathetic and parasympathetic indices towards more "normal" middle region of the reference values. These studies show that yoga has a great potential to improve our physiological functions, psychosomatic health and overall performance.

1.4. EPIDEMIOLOGY OF CARDIO VASCULAR DISEASE

Coronary artery disease (CAD) is a leading cause of death in the western world. It is responsible for one-third of all global deaths. Nearly 85% of the global mortality and disease burden from CAD is borne by low and middle income countries. In India, approximately 53% of CAD deaths are in people younger than 70 years of age; in China, the corresponding figure is 35%. The majority of the estimated 32 million heart attacks and strokes that occur every year are caused by one or more cardiovascular risk factors like hypertension, diabetes, smoking, high levels of blood lipids, physical inactivity and most of these CAD events are preventable if meaningful action is taken against these risk factors(WHO Report 2002).

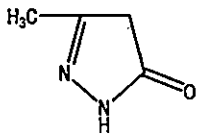
Ischemic heart disease (IHD) is a major cause of death in industrialized countries and is rising at an alarming rate in many developing countries. Coronary artery disease (CAD) is a leading cause of death in the western world. It is a leading cause of mortality and is responsible for one third of all global deaths. Nearly 85% of the global mortality and disease burden from CVD is borne by low and middle income countries. According to the World Health Organization projections, it is predicted that morbidity and mortality

due to cardiovascular diseases in India will reach an epidemic proportions by the middle of this century due to rapid changes in life style and a significant segment of which will be due to ischemic heart disease In India approximately 53% of CVD deaths are in people younger than 70years of age. By 2020 CVD will be the leading cause of death in developing countries like India (WHO, 2007).

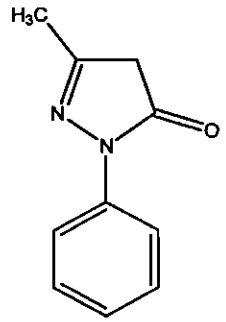
Awareness of the rising incidence of IHD in India coupled with prohibitive cost of treatment particularly for developing country generated urgency for the rapid development of a novel drug molecule with fewer side effects in the amelioration of IHD. Thus the present study is emphasized to synthesize some novel molecule by using pyrazolone as a heterocyclic scaffold and to evaluate the cardioprotective effect of pyrazolone derivatives since it has been proved that these molecules have preventive effects on myocardial injury following ischemia and reperfusion in the rat heart (Yangisawa *et al.*, 1994) and in patients with acute myocardial infarction (Tsujita *et al.*, 2004).

1.5. PYRAZOLONE DERIVATIVES IN ISCHEMIC INJURY

Pyrazolone was first synthesized in 1887 as antipyrine to reduce fever. It is a key structure in numerous compounds of therapeutic importance. The drugs containing pyrazolone nucleus are known to display diverse pharmacological activities such as antibacterial, antifungal, anti-inflammatory, analgesic and antipyretic activities. The compounds like 3-Alkyl-4-arylmethylpyrazol -5-ones are reported to exhibit potent antihyperglycemic activity while 1-phenyl-3-tetrafluoroethylpyrazol- 5-one is an anxiolytic. Thus, the biological activities of pyrazol-5-ones depend on the nature of the substituent. For instance Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a strong novel free radical scavenger is used for treatment of patients with acute brain infarction. It has preventive effects on myocardial injury



3-methyl pyrazol-5-one



3-methyl-1-phenyl pyrazol-5-one(Edaravone)

following ischemia and reperfusion in patients with acute myocardial infarction. The antioxidant actions of edaravone include enhancement of prostacyclin production, inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals, inhibition of alloxan-induced lipid peroxidation, and quenching of active oxygen, leading to protection of various cells, such as endothelial cells, against damage by reactive oxygen species (ROS). Recently, it has been proved that edaravone improves endothelial function through a decrease in ROS in smokers. From a clinical perspective, it is important to select an appropriate drug that is effective in improving endothelial function in patients with cardiovascular diseases. The novel free radical scavenger edaravone may represent a new therapeutic intervention for endothelial dysfunction in the setting of atherosclerosis, chronic heart failure, diabetes mellitus and hypertension. This study focused on clinical findings and on putative mechanisms underlying the beneficial effects of the anti oxidative agent i.e edaravone on the atherosclerotic process in patients with cardiovascular diseases.

Pyrazolone derivatives have been used in patients with acute brain infarction since April 2001 in Japan (Otomo, 2003). These derivatives have been shown to be effective against brain edema after ischemia and reperfusion injury in animal models (Nishi et al., 1989) and in stroke patients (Toyoda *et al.*, 2004). Several lines of evidence have demonstrated that oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases, including hypertension, hypercholesterolemia, diabetes mellitus, atherosclerosis, myocardial infarction, angina pectoris and heart failure (Chien, 2009).

The lipid peroxidation is found to cause formation of atherosclerotic plaques, neurological disorders, cancer, diabetes mellitus, myocardial infarction and ageing. It is associated with ischemia-reperfusion injury and hyperoxic lung injury. The peroxides derived from lipid peroxidation such as MDA (TBARS) and 4-HNE have been strongly associated with myocardial ischemic reperfusion injury (Blasig *et al.*, 1995; Ski et al., 2008). It is expected that pyrazolone derivatives have beneficial effects on coronary artery and myocardial cells after ischemic and post ischemic myocardial injury in patients with ischemic heart diseases, including acute myocardial infarction and angina pectoris. Some animal studies using acute myocardial ischemia-reperfusion models have suggested the protective effects of pyrazolone derivatives on myocardial damage.

From the above mentioned facts and figures, it can be ascertained that the oxidative stress plays an important role in the etiopathogenesis of atherosclerosis and ischemic heart disease. Previous studies have shown that compounds like pyrazolone derivatives played a vital role in the above mentioned diseases. It may be hypothesized that the overall beneficial effects of pyrazolone derivatives in ischemic heart disease may be due to their protective properties by either acting as an anti oxidant itself or by increasing the endogenous anti oxidant levels. There have been a few studies, which have indicated the cardioprotective potential of the same. Since limited studies have been conducted in this context, the present study is in sighted to elucidate the cardio protective property of pyrazolone derivatives in terms of plasma lipid profile, myocardial injury markers and induction of endogenous anti oxidant enzymes in myocardial ischemic reperfusion injury. Hence forth the present study intends to concentrate on the mechanism of cardioprotective action of pyrazolone derivatives in myocardial IR injury.

1.6. EVALUATION OF CARDIO PROTECTIVE PROPERTIES

Cardio protective action can be evaluated by

i) Chemical methods

(a) Isoproterenol induced myocardial ischemic injury model.

(b) Doxorubicin induced myocardial ischemic injury model.

ii) Instrumental method using Langendorff isolated-perfused heart model.

Isoproterenol (ISO) is a ß-adrenergic agonist that causes severe stress in myocardium resulting in the infarct like necrosis of heart muscle (Welexer, 1978). Some of the mechanisms proposed to explain ISO induced damage in cardiac myocytes include hypoxia due to myocardial hyperactivity, coronary hypotension, calcium overload, depletion of energy reserves and excessive production of free radicals due to oxidative metabolism of catecholamine's (Mohanty *et al.*, 2004). ISO induced myocardial necrosis in rats is a widely used experimental model for evaluation of cardioprotective effect of various herbal drugs (Naik and Panda, 2008; Nandave *et al.*, 2009), because pathophysiological changes following ISO administration in rats are comparable to those taking place during myocardial injury in

humans (Nirmala and Puvanakrishnan, 1996). Therefore, it is a suitable model to study myocardial ischemic injury.

Doxorubicin an anthracycline is well established and highly efficacious drug in the fight against many kinds of cancers like solid tumors, leukemia, soft tissue sarcoma, breast cancer, small cell carcinoma of the lung and esophageal carcinomas (Blum and Carter, 1974; Chabner *et al.*, 2001).But, its clinical usefulness is still restricted due to its specific toxicities to cardiac tissue. Congestive heart failure, cardiomyopathy and electrocardiographic changes were demonstrated after cumulative doxorubicin administration (Lenaz and Page, 1976).The mechanisms proposed for cardio toxic effects of doxorubicin includes free radical induced myocardial injury, lipid peroxidation (Myers *et al.*, 1977),mitochondria damage (Bier and Jaenke, 1976),decreased gravity of Na^+K^+ATPase, vasoactive amine release(Bristow *et al.*, 1980), impairment in myocardial adrenergic signaling/regulation, increase in serum total cholesterol, triglyceride and low density lipoproteins (Ilskowic and Singal, 1997).Generation of reactive oxygen species like superoxide anion and hydrogen peroxide by doxorubicin leads to causing impairment of cell functioning and cytolysis (Daoud, 1992).Due to the presence of less developed antioxidant defense mechanisms, heart is particularly vulnerable to injury by anthracycline induced reactive oxygen species.

Liberation of free radicals is central to the mechanism of doxorubicin induced damage to the myocardium (Potemski *et al.*, 2006).It also causes the elevation of serum enzymes like lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) (Abd-Alah *et al.*, 2002).Endogenous antioxidant deficits have been suggested to play a major role in doxorubicin induced cardiomyopathy and heart failure (Hanaa *et al.*, 2005). Hence it is also suitable model to evaluate myocardial injury

The concept of the isolated perfused heart system was introduced and established by Oskar Langendorff in 1895. It is now a predominant technique in pharmacological and physiological research. The technique allows the examination of cardiac contractile strength (inotropic effects), heart rate (chronotropic effects) and vascular effects without the neuronal and hormonal complications of an intact animal model. (Frederich *et al.*, 2005). The technique originally required elevated reservoirs to provide a constant, gravity supplied pressure but the technique and equipment have evolved to include both

constant pressure and constant flow models in both recirculating and non-recirculating modes.

After a midline sternotomy, the hearts were rapidly excised and perfused retrogradely at a constant perfusion pressure of 80 mmHg with a modified Krebs' solution containing NaCl (120 mM), $NaHCO_3$(25mM), $MgSO_4$(1.2mM), KH_2PO_4(1.2 mM), $CaCl_2$ (1.2 mM), and glucose (11 mM). The perfusate buffer was saturated with a 95% O_2 and 5% CO_2 gas mixture at 37^0C. In the Langendorff preparation, the aorta is cannulated and the heart is perfused in a retrograde (reverse) fashion, usually with a nutrient rich, oxygenated solution. The pressure of the solution causes the aortic valve to shut and the perfusate is then forced into the ostium and into the coronary vessels. This allows the heart to beat for several hours. Then the heart is subjected to evaluate the cardio protective effect as per the experimental protocol.

Cardio vascular diseases have been linked to oxidative stress which is initiated by the reaction of free radicals with biological macromolecules such as proteins, lipids and DNA (Martinez-Cayuela, 1995). The serum enzymes namely LDH, AST, ALT and ALP serve as sensitive indices to assess the severity of myocardial infarction (Sheela-Sasikumar *et al.*, 2000). The increased activities of these enzymes following injection of ISO as observed in this study confirmed the onset of myocardial necrosis (Parithaithayarasi *et al.*, 1997).

Most tissue damages are mediated by free radicals which attack membranes through peroxidation of unsaturated fatty acids (Stringer *et al.*, 1989). Myocardial injury induced by ISO in rats results in increased lipid peroxidation, which is an evidence of intensified free radical production (Lefer and Granger, 2000). These free radicals initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity. In this study, malondialdehyde (MDA) level was elevated in the heart of myocardial injured rats which is an indication of oxidative stress in the tissue. Recently another lipid peroxidation marker 4- HNE has been proposed as an important marker of radical induced myocardial injury (Blasig *et al.*, 1995).

Endogenous antioxidant enzymes such as SOD, CAT, GS peroxidase (GPX) and GST are the first-line cellular defense against oxidative stress, decomposing oxygen and H_2O_2

before they interact to form the more reactive hydroxyl radical (OH). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. SOD and CAT are important antioxidant enzymes in mitigating free radical-induced cell injury. A decrease in the activity of SOD and CAT can result in the decreased removal of superoxide ion and H2O2 radicals that brings about a number of reactions, which are harmful to myocardium. Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. An increase in SOD activity is beneficial in the event of increased free radical generation. However, it has been reported that a rise in SOD activity, without a concomitant rise in the activity of CAT and/or GPX, may be detrimental because SOD generates H_2O_2 as a metabolite, which is more cytotoxic than oxygen radicals, and must be scavenged by CAT or GPX (Yim *et al.*, 1990). Thus, a simultaneous increase in CAT and/or GPX activity is essential for an overall beneficial effect of an increase in SOD activity (Harman, 1991). The endogeneous anti oxidative enzyme Glutathione (GSH) is an important antioxidant which plays the role of an intracellular radical scavenger and is a substrate for many xenobiotic elimination reactions. Decreased level of GSH observed in the heart tissue may be a result of increased oxidative stress. Glutathione has the ability to manage oxidative stress with adaptional changes in enzymes regulating its metabolism (Arulselvan and Subramanian, 2007).

The decreased activities of SOD and GR in the heart as observed in this study may be due to increased production of reactive oxygen radicals which are capable of reducing the activities of these enzymes (Basheeruddin Asdaq and Prasannakumar, 2009). SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals (Lin et al., 2005) while GR is required for the conversion of oxidized (GSSG) to reduced (GSH) glutathione (Zhang and Tan, 2000).

Lipid peroxidation is associated with ischemia-reperfusion injury and hyperoxic lung injury. The peroxides derived from lipid peroxidation such as thiobarbituric acid reactive substances (TBARS) and hydroxyl 2-non-enal (4-HNE) has been strongly associated with myocardial ischemic reperfusion injury (Blasig et al., 1995: Ski Kami *et al.*, 2008). Based on the above reports, the cardio protective activity will be evaluated by estimating the serum lipid profile, serum marker enzymes, and myocardial tissue endogenous markers and anti oxidant parameters.

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