Literature Review
2.0 Background

Cardiovascular diseases account for 12 million deaths annually worldwide, particularly Myocardial infarction. In terms of global burden of disease in 1999, the World Health Organization (WHO) placed myocardial infarction in sixth place and stroke in seventh place, but by 2020 it is estimated that these heart diseases would have occupied first and fourth place respectively\(^7\). It continues to be a significant problem in industrialized countries and is becoming an increasingly significant problem in developing countries\(^3\). Asians, including Indians appear to be at a higher risk of developing heart attack\(^7\). It forms the major cause of mortality in the middle age and elderly population\(^8\).

Myocardial infarction (MI) is the rapid development of myocardial necrosis caused by a critical imbalance between oxygen supply and demand of the myocardium. This usually results from plaque rupture with thrombus formation in a coronary vessel, resulting in an acute reduction of blood supply to the myocardium\(^3\). While there are many reasons for a heart attack to occur, the most common causes include the inability of the heart to pump the adequate level of blood or when enough blood does not reach the heart. Studies show that heart attack risk increases on obesity, atherosclerosis, hypercholesterolemia, hypertriglyceridemia, Diabetes mellitus, smoking, family history, sex, sedentary lifestyle, certain diabetes drugs and lung disease inhalers, etc\(^1\).

MI is the most dreaded sequel among ischemic heart diseases, which is invariably followed by several biochemical alterations, such as lipid peroxidation, free radical damage, hyperglycemia, hyperlipidemia etc. leading to qualitative and quantitative alterations of the myocardium\(^2\).

Recent trend is to recommend herbal treatment for cardiovascular diseases, particularly in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia\(^6\). This is because since the beginning of human civilization, herbs have...
been an integral part of society, valued for both their culinary and medicinal properties.

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches from herbs, as they are free from side effects caused by synthetic chemicals. They are used either as preventive or as curative throughout the world. WHO estimates that about three-quarters of the world’s population currently use herbs and other forms of traditional medicines to treat their diseases. There is a great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lesser costs.

2.1 Biochemical Studies

2.1.1 Myocardial Markers

The early recognition of cardiac ischemia and accurate placement of the patient in the risk spectrum of the acute coronary syndrome are critical in patients with acute myocardial infarction. Apart from clinical history, physical examination and accurate ECG interpretations, cardiac biomarkers are equally valuable in the initial evaluation of patients with non-traumatic chest pain. Measurement of certain enzymes in serum has been essential for the evaluation of acute myocardial infarction since the first reports of elevated serum glutamate oxaloacetate transaminase (SGOT). Over the past two decades, testing for increased serum creatine kinase (CK) and lactate dehydrogenase (LDH) has become routine to access myocardial infarction.

Enzymes are essentially harbored inside the cells of their origin and restrained within the plasma membrane. As long as the integrity of the plasma membrane is maintained the enzymes do not leak out of the cell. This integrity is maintained by the cell’s ATP production. ATP production of the cell can be hampered in many ways, such as the lack of oxygen carrying capacity and blood supply; treatment with
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chemicals and drugs and other environmental pollutants, extreme physical stress such as heat, radiation; exposure to microbial agents and subsequent infection; disruption or malfunction of the immune system; genetic defects leading to metabolic disorders and nutritional disorders. One or many of such distress will cause the plasma membrane to deteriorate. During the early stages of this loss of integrity there is an efflux of potassium ions and an increased influx of sodium ions. This leads to increased water retention in the cell leading to swelling. In later stages, calcium influx occurs which acts as a stimulus to the intracellular enzymes leading to their hyperactivity and an increase in cell damage and disruption of the cell membrane. Finally, all these processes lead to an increased production of free radical and oxidative damage and the membrane become leaky and molecules of all sizes eventually leak out depending on the extent of damage.

Figure 3: Release of Cardiac Marker Enzymes.

ALT and AST are members of the transaminase family of enzymes. ALT and AST are sometimes called serum glutamate pyruvate transaminase (SGPT) and serum...
glutamate oxaloacetate transaminase (SGOT), respectively. ALT catalyses the transfer of amino groups of L-alanine and glutamate. AST catalyses the transfer of amino and keto groups between alpha-amino acids and alpha-keto acids thereby acquiring the name transferase\textsuperscript{92}. The activities of both AST and ALT are high in tissues especially liver, heart, and muscles. In myocardial infarction high activity of AST is seen in serum. ALT activity is within normal range or slightly increased in uncomplicated myocardial infarction. Rise in AST is seen within 6 to 8 hours of the onset of chest pain, highest level at 18 to 24 hours and returns to pre infarction levels by 4th to 5th day. There are other superior markers available for myocardial infarction as AST lacks the tissue specific characteristics, as its activity may also be increased in diseases of other tissues like liver and skeletal muscles\textsuperscript{90}.

LDH catalyses the reversible reaction between pyruvic and lactic acids. It is present in nearly all types of metabolizing cells. The enzyme is especially concentrated in heart, liver, red blood cells, kidneys, muscles, brain and lungs. Certain diseases have elevated LDH levels. One of the most important diagnostic uses for the LDH is in the diagnosis of myocardial infarction or heart attack. The total LDH level rises within 24-48 hours after a heart attack, peaks in two to three days and returns to normal in five to ten days\textsuperscript{93}.

Creatine kinase is an enzyme in heart, brain and skeletal muscle that catalyses the reaction that provides high-energy phosphate early in the anaerobic phase of organ function. CK activity in serum invariably increases after myocardial infarction (MI). The levels rise within 3 to 6 hours after a heart attack. If there is no further damage to the heart muscle, the level peaks at 12 to 24 hours and returns to preinfarction level in 12 to 48 hours. Preinfarction values of CK are usually less than 6%, but following an infarction values can increase up to 30% depending on the extent of myocardial damage, location of the infarct or the methods used for analysis\textsuperscript{90}.
Changes in the activities of these cardiac marker enzymes can be treated with various drugs including herbs and herbal extracts, isolated bioactive compounds from medicinal plants as well as Ayurvedic and Siddha formulations. Herbal extract have long been recognized to possess many properties including antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic effects.

Studies on few herbs, herbal extracts, formulations as well as active principles that could be of use in the treatment of myocardial infarction were reviewed and presented in sequel.

Suchalatha et al. studied the cardioprotective effect of *Terminalia chebula* Retz. in the experimental model of myocardial injury. Isoproterenol treated animals exhibited significant elevation in the activity of AST, ALT, CK and LDH in serum with a concomitant reduction in the heart tissue compared to the normal control. Ethanolic extract of *Terminalia chebula* (500mg/kg body) pretreatment significantly reduced the activity of the marker enzymes when compared with isoproterenol-administered rats.

The efficacy of aqueous extract of *Centella asiatica* L. on adriamycin induced myocardial failure in rats was studied by Gnanaparagasam et al. Activities of marker enzymes of cardiac function (GOT, GPT, CPK and LDH) lowered in the heart of rats given ADR as compared with those of the control rats. The changes in the activities of these enzymes due to ADR administration were prevented to near normal in rats, which were pre-co-treated with *Centella asiatica*.

Rajaprabhu et al. proved the protective effects of *Picrorhiza kurroa* Royle ex Benth, an ayurvedic medicinal plant, on myocardial defense in adriamycin-induced cardiomyopathy in rats. Intraperitoneal administration of adriamycin (1.5 mg/kg body weight/day, i.p. for 15 days) caused significant rise in the levels of diagnostic marker enzymes (ALT, ASLT, CPK and LDH) in plasma. Concomitant decline in the level of...
these diagnostic marker enzymes were observed in the animals treated with *P. kurroa* ethanolic extract (50 mg/kg body weight/day for a period of 15 days).

The alcoholic extract of latex obtained from *Calotropis procera* (Aiton) W. T. Aiton was evaluated by Ahmed Muenen et al. for protection against Isoproterenol induced myocardial infarction. Elevated levels of the marker enzymes such as CK-MB, LDH, SGOT and SGPT in serum indicated the heart damage induced by Isoproterenol. Pretreatment with an alcoholic latex extract of *Calotropis procera* at a dose of 300mg/kg body wt., administered orally thrice a day for 30 days, reduced significantly the elevated marker enzyme levels in the serum.

Raju et al. tested the cardioprotective potential of *Momordica cymbalaria* Fenzl against Isoproterenol-induced myocardial injury. Pretreatment with ethanolic extract of *M. cymbalaria* at the dose levels of 250 and 500mg/kg prevented the elevation of serum marker enzymes, LDH, CK-MB, AST and ALT and uric acid levels caused by Iso-induced myocardial injury. The protective effect was more prominent at 500 mg/kg.

The protective effects of the aqueous extracts of the leaves of *Cichorium intybus* L. have been examined in the ageing myocardium of albino rats during Isoproterenol (ISO) induced myocardial injury. Changes occurring in lactic dehydrogenase (LDH) activity were measured in the serum and myocardium of albino rats, as LDH is an important biochemical marker of confirming myocardial infarction. The activity of myocardial LDH was studied in 21 days; 5 months and 18 months old control and ISO administered albino rats. A significant suppression of LDH activity was noted in all the age groups on inducing myocardial ischemia. The decline in the cardiac LDH activity was proportional to the elevation of serum LDH in all the age groups examined. Aged rats exhibited comparatively greater loss of cardiac LDH activity. The study by Nayeemunnisa et al. proved that dietary supplement of *Cichorium intybus* (500mg/kg for 10 days) reduced the loss of LDH.
activity thereby rendering the myocardium comparatively more efficient in the aged rats.

Muralidharan\textsuperscript{101} \textit{et al.} studied Cardioprotective effect of \textit{Daucus carota} L. on Isoproterenol-induced myocardial infarction. \textit{Daucus carota} Linn. tubers were extracted with water and analyzed for its inotropic and cardioprotective effects by measuring various biochemical parameters at the test doses of 250 and 500 mg/kg. Estimating serum aspartate transaminase, alanine transaminase and lactate dehydrogenase levels assessed cardioprotection. The levels altered by isoproterenol induction were restored significantly by the administration of the extract. The result of the study implies that \textit{D. carota} is a potential source to protect heart from myocardial infarction and to maintain its tonicity.

Velavan\textsuperscript{102} \textit{et al.} evaluated the cardioprotective effect of \textit{Vitis vinifera} L. seed against Isoproterenol induced myocardial ischemia. Normal Wistar strain rats were pretreated with ethanolic extract of \textit{Vitis vinifera} seed (500mg/kg body weight) for 28 days and then intoxicated with isoproterenol (ISO) (20mg/100g, i.p. for 2 consecutive days). The activities of cardiac marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) were decreased in heart with concomitant increase in plasma. The prior administration of \textit{Vitis vinifera} significantly prevented the isoproterenol-induced alterations and restored the cardiac markers.

Mini\textsuperscript{103} \textit{et al.} studied the cardioprotective effect of coconut kernel protein in Isoproterenol administered rats. Administration of Isoproterenol resulted in increased CPK, SGOT and SGPT activity in the serum, as compared to normal rats. In coconut protein fed rats, the activity was restored to near normal status. The results indicate that consumption of coconut protein may cause less incidence of myocardial infarction.
Administration of fish oil to human has been recommended as a preventive or therapeutic approach for the treatment of cardiovascular disease, based on its ability to reduce both the thrombotic risk and the level of circulating atherogenic lipids. Padma et al. proved it in Isoproterenol induced myocardial necrosis animal model. Serum LDH, CK and transaminases showed significant increase in their activity in Isoproterenol administered rats when compared to normal rats. Increase in serum uric acid was also observed. Fish oil treated animals showed decreased activities of these marker enzymes and a reduction in uric acid level in Group IV (Fish oil + Isoproterenol) animals.

The study investigated by Prabhu et al. dealt with the protective role of mangiferin, a polyphenol from Mangifera indica Linn. (Anacardiaceae). Myocardial damage caused by Isoproterenol was determined by the increased activity of serum LDH and CK-MB and increased uric acid level. Upon treatment with mangiferin (100 mg/kg body weight suspended in 2ml of dimethyl sulphoxide) given intra peritoneally for 28 days restored the near normal status of the cardiac marker enzymes.

Farvin et al. designed a study to examine the effects of squalene on tissue defense system in Isoproterenol-induced myocardial infarction. Activities of diagnostic marker enzymes ALT, AST, LDH and CPK in the heart tissue of experimental groups of rats were determined. The prior administration of squalene at 2% level along with feed for 45 days significantly prevented the Isoproterenol induced elevation in the activities of diagnostic serum marker enzymes in plasma of experimental animals.

Senthil et al. proved the protective effects of ursolic acid (UA) against myocardial ischemia induced by isoproterenol in rats. Normal Wistar strain rats were pretreated with UA (20, 40 and 60 mg/kg, s.c) for 7 days and then intoxicated with isoproterenol (85 mg/kg, s.c.) for 2 consecutive days. Animals were assessed for the activities of cardiac markers (AST, ALT, LDH and CPK). The prior administration of
UA significantly prevented the Isoproterenol-induced alterations and restored the enzymes to near normal.

The results of the study by Karthick et al. showed that rutin, a bioflavonoid possesses cardioprotective activity against Isoproterenol induced myocardial infarction. Isoproterenol administration showed a significant increase in the activity of serum marker enzymes (CK, LDH, AST and ALT) and a significant decrease in the activity of these enzymes in the heart. Pretreatment with rutin (40 or 80 mg/kg) to Isoproterenol-treated rats orally for a period of 42 days caused a significant effect.

Betaine (N, N, N-trimethyl glycine) distributed widely in animals, plants, and microorganisms and rich dietary source include seafood, especially marine vertebrates; wheat germ or bran; and spinach. Prior oral treatment with betaine significantly prevented the isoprenaline-induced elevation in the levels of diagnostic marker enzymes (ALT, AST, LDH and CPK) in plasma of experimental animals in a study conducted by Ganesan et al.

Chitosan, a polymer of β-(1-4)-D-glucosamine, is chemically similar to that of the plant fiber, cellulose. It is one of the most abundant naturally occurring polysaccharides present in the shellfish, clams, krill, oysters, squid, fungi and insects. Preventive effects of chitosan, on isoprenaline induced myocardial infarction in male albino rats is proved in a study conducted by Sivakumar et al. The study further proved that the dietary supplementation of 2% chitosan for 60 days significantly reduced the isoprenaline-induced elevation in the levels of plasma diagnostic marker enzymes to near normal.

Kumar et al. investigated the protective effect of glutamine in isoprenaline-induced myocardial infarction. Injection of isoprenaline caused significant increase in the levels of diagnostic marker enzymes in plasma and a parallel decline in heart tissue was also observed. Prior oral administration of glutamine significantly prevented the isoprenaline induced adverse effects and maintained the activities of the
marker enzymes at near normalcy. The cardioprotecive effect of glutamine is probably because of strengthening of the myocardial membrane by its membrane stabilizing action.

Carnitine (L-3-hydroxy-4-trimethylammonium butyrate), which plays an important role in the transmembrane transport of long chain fatty acids for their oxidation in the mitochondria, has been reported to offer protection against myocardial infarction induced by Isoproterenol in a study conducted by Kumari and Menon. Levels of diagnostic marker enzymes GOT, GPT and CPK were decreased in the serum in carnitine-treated rats compared to the levels in isoprenaline-treated controls.

Sudhira et al. investigated the cardioprotective effect of amlodipine in oxidative stress induced by experimental myocardial infarction in rats. Adrenaline was administered and myocardial damage was evaluated biochemically (significantly increased serum AST and LDH) and histologically. Amlodipine was administered as pretreatment for 14 days in adrenaline treated rats. Statistically significant amelioration in biochemical parameters supported by significantly improved myocardial morphology was observed in amlodipine-pretreated rats.

The study designed by Nazam Anzari et al. was to investigate the effect of oral curcumin pretreatment (200 mg/kg) on isoproterenol induced myocardial injury in rats. Isoproterenol (85 mg/kg, s.c., in two divided doses at 24 h intervals) administration induced a statistically significant increase (P < 0.01) in serum lactate dehydrogenase, creatine kinase, aspartate transaminase, and alanine transaminase activities as compared to vehicle control rats. Curcumin (200 mg/kg) pretreatment for 20 days significantly lowered (P < 0.01) the serum lactate dehydrogenase, creatinekinase, aspartate transaminase, and alanine transaminase activities in isoproterenol treated rats.
Senthil\textsuperscript{115} \textit{et al.} proved the protective effect of oleanolic acid (OA) against isoproterenol-induced myocardial ischemia in rat myocardium. Wistar strain rats were pretreated with OA (20, 40, and 60 mg/kg, s.c) for 7 days and then intoxicated with isoproterenol (ISO, 85 mg/kg, sc for 2 consecutive days). Heart were excised from the experimental animals and assessed for the activities of marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK). Leakage of cardiac markers confirmed the severe myocardial damage occurring as a consequence of isoproterenol-induced ischemia, and they also showed the significant improvement followed by oleanolic acid pretreatment. These findings provided evidence that oleanolic acid was found to be protecting rat myocardium against ischemic insult and the protective effect could be attributed to membrane-stabilizing action of oleanolic acid.

Rao \textit{and Vishwanath} \textsuperscript{116} studied the cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats. Ischemia-reperfusion resulted in significant cardiac necrosis, which is indicated by elevated levels of serum marker enzymes such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, lactate dehydrogenase, and creatine kinase. Silymarin was administered orally to Wistar albino rats (200 g to 250 g) at three different doses (100 mg/kg, 250 mg/kg and 500 mg/kg), by gastric gavage for one week significantly reduced the activities of serum marker enzymes to near normal status.

‘Abana’, a herbomineral preparation, showed significant protection against the biochemical changes induced by isoproterenol in rats in a study conducted by Tandon \textsuperscript{117} \textit{et al.} In myocardial necrosis, the increased levels of serum creatine phosphokinase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and \(\gamma\)-glutamyl transpeptidase were reversed on Abana treatment. This showed that Abana treatment could contribute in restoring myocardial integrity and cardiac function disturbed by isoproterenol-induced ischemia.
Naik and Panda\textsuperscript{118} studied the antioxidant activity of A.V. Circulo (AVC), a polyherbal formulation in isoproterenol (ISO)-induced oxidative stress in rats and attempts were made to correlate its cardioprotective activity with antioxidant activity. Myocardial necrosis was produced in rats with ISO (85 mg/kg, s.c.), injected twice at an interval of 24 h. Daily pretreatment of AVC (500 mg/kg) for 21 & 45 days to rats which were treated with ISO on the last 2 days, resulted in a significant cardioprotective activity as reflected in the decreased levels of serum marker enzymes (AST, ALT, LDH and CK) when compared to isoproterenol treated rats. The study concluded that AVC (500 mg/kg) oral treatment for 45 days showed greater cardiac protection.

\textbf{2.1.2 Free Radicals, Antioxidants and Oxidative Stress}

The story of free radicals started more than two centuries ago. It was Lavoisier\textsuperscript{119} who made the first observation that oxygen has two main effects, i.e. it supports life but it also has toxic side effects. The discovery of superoxide dismutase by McCord and Fridovich\textsuperscript{120} inspired biologists and clinicians around the world to study the role of free radicals in biology and medicine. Free radicals and related species have attracted a great deal of attention in recent years. During the last few decades, research data has prompted a passionate debate as to whether oxidation, or specifically, oxidative stress mediated by free radicals/reactive oxygen species (ROS)/reactive nitrogen species (RNS), is a primary or secondary cause of many chronic diseases\textsuperscript{121}.

Reactive oxygen/nitrogen species (ROS/RNS) and free radicals are constantly formed in the human body and removed by an antioxidant defense system. A certain amount of ROS/RNS production is, in fact, necessary for proper health; for example ROS/RNS help the immune system to eliminate microorganisms, which is clearly exemplified in patients with granulomatous disease\textsuperscript{122}. In healthy individuals, the generation of ROS/RNS appears to be approximately in balance with antioxidant defense.
2.1.2.1 ROS/RNS

Free radicals and ROS/RNS species of importance in living organisms include molecules such as superoxide anion (\(\cdot O_2^-\)), hydroxyl radical (\(\cdot OH\)), nitric oxide (nitrogen monoxide \(\cdot NO\)), nitrogen dioxide (\(\cdot NO_2\)) and lipid radicals. Other ROS / RNS, such as hydrogen peroxide (\(H_2O_2\)), peroxynitrite (\(ONOO^-\)), peroxynitrous acid (\(ONOOH\)), Singlet oxygen (\(1^1O_2\)), Ozone (\(O_3\)) and hypochlorous acid (\(HOCl\)), are not free radicals but have oxidizing effects that contribute to oxidative stress. The term 'reactive oxygen species' (ROS) and 'reactive nitrogen species' (RNS) is a collective term that includes not only the radicals but also the non-radicals.

The cellular production of one ROS may lead to the production of several others via radical chain reactions. Many ROS / RNS possess unpaired electrons and thus are free radicals. The production of free radicals occurs either by the addition or by the removal of an electron in an oxidation/reduction reaction. Since oxygen has two electrons in its outermost shell and is a diradical, it requires four electrons to be completely reduced to water\(^{123}\). Oxygen is also the terminal acceptor of four electrons associated with the production of high-energy phosphates. However an addition of one electron at a time results in the formation of reactive oxygen species\(^{124}\).

In the univalent reduction pathway, the addition of one electron to molecular oxygen results in the production of superoxide anion radical (\(\cdot O_2^-\)). Addition of one electron to \(\cdot O_2^-\) results in the formation of hydrogen peroxide (\(H_2O_2\)). The \(H_2O_2\) is not a radical by itself, but is capable of causing cell damage by interacting with transition metal ions such as iron. A single electron reduction of \(H_2O_2\) results in the formation of hydroxyl radical (\(\cdot OH\))\(^{125}\). The \(\cdot OH\) is a highly reactive molecule with an extremely short half-life, and therefore has a very limited diffusion capacity. Finally, the addition of a fourth electron results in the formation of water. The first excited state of oxygen i.e. a singlet oxygen (\(1^1O_2\)), can also initiate oxygen radical chain reactions.
As new biological aspects of •NO have been discovered, speculation has emerged that this radical plays important roles in various pathophysiological mechanisms. •NO is produced by nitric oxide synthase (NOS); catalyze the oxidation of L-arginine to L-citrulline. •NO forms RNS by reacting with •O2⁻, molecular oxygen, and metal complexes. •NO rapidly reacts with •O2⁻ to give rise to ONOO⁻. Its protonated form peroxynitrous acid (HNOOH) is also extremely reactive. Nitrogen dioxide •NO2 is a strongly oxidizing radical, which is formed from ONOO⁻. Other important products derived from •NO are dinitrogen trioxide (N₂O₃) which forms in the presence of molecular oxygen and S-nitrosothiols (RSNO) that form by nitrosation of thiols.

2.1.2.2 Sources of Free Radicals

Oxidative stress in human biology comes from a variety of sources. Mitochondrion is the major source of •O2⁻. Activated neutrophils, and cytoplasmic enzymes such as Xanthine oxidase, NADPH oxidase are also the sources of •O2⁻. Macrophages and neutrophils also produce ROS such as H₂O₂ and HOCl as a means of bacterial killing. Myeloperoxidase (MPO) is a heme containing protein and the only human enzyme known to generate HOCl.

ROS may be generated within the membranes, in association with the arachidonic acid cascade and with the auto-oxidation of catecholamines. Iron containing dioxygenases, lipoxygenases, are prooxidant enzymes, which catalyze the insertion of molecular oxygen into polyunsaturated fatty acids to produce biologically active lipids such as prostaglandins, thromboxanes and leukotrienes. Drugs may exert toxic effects by promoting ROS formation during their metabolism, e.g. cardiomyopathy associated with daunomycin and doxorubicin. Smoking represents a major threat to health and many of its damaging effects can be attributed to its free radical content and a subsequent oxidative damage.
Figure 4: Sources of ROS in Vascular Cells. [Adapted from: Madamanchi NR, Vendrov A, Runge MS. Oxidative Stress and Vascular Disease. Arterioscler Thromb Vasc Biol 2005; 25: 29-38]

NOS is a family of enzymes with three different isoforms. Two isoforms, neuronal and endothelial are constitutively expressed while one is induced in response to cytokines and endotoxins among other stimuli. In the context of cardiovascular system, endothelial NOS (eNOS; NOS3) and inducible NOS (iNOS; NOS2) are most relevant.

2.1.2.3 Free Radical Mediated Cell Injury

Oxidative stress is the term referring to the imbalance between generation of reactive oxygen species and the activity of the antioxidant defenses. These cause excessive production of ROS/RNS and loss of antioxidant defenses. The excess species react with all classes of biomolecules, including lipids, proteins, and nucleic
acid bases which can severely affect cell structure and viability. Especially cellular targets such as thiols, proteins and lipids, many of them which have special roles for cellular signaling, are affected by increased ROS /RNS.

Figure 5: Free Radical Mediated Cardio Myocyte Injury [Adapted from: Doğan Yücel, Mehmet Şeneş, Çığdem Topkaya, Oğuzhan Zengi. Oxidative / Nitrosative Stress in Chronic Heart Failure: A Critical Review. Turk J Biochem 2006, 31 (2), 86-95]

Free radical–induced lipid peroxidation has been suggested to alter membrane structure and function. The lipid peroxidation process is initiated by the removal of a hydrogen atom from the unsaturated site in a fatty acid resulting in the production of a lipid radical. The peroxidation of lipids is known to cause alterations in membrane fluidity. There is also evidence suggesting that free radicals can modify the protein structure and function. In this regard, proteins rich in sulphhydryl compound, has been used to characterize radical-induced damage. Oxidation of the sulphhydryl groups in proteins results in the formation of toxic thiol compounds.
In the myocardium, oxygen radicals have been shown to affect \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger, \( \text{Na}^+ - \text{K}^+ \text{ATPase} \) and \( \text{Ca}^{2+} \text{ATPase} \) activities. Consequently, in conditions of increased ROS /RNS production, a variety of cellular responses through generation of secondary reactive products result in cell death by necrosis or apoptosis.

### 2.1.2.4 Antioxidants

Human body controls and survives the continuous ROS production due to a delicate balance between cellular systems generating the various oxidants and those maintaining the antioxidant defense mechanisms. There are enzymic and non-enzymic antioxidant defense mechanisms, which helps in maintaining the antioxidant status.

Enzymic defense mechanisms include the enzymes superoxide dismutase (SOD), catalase, glutathione reductase (GR), and glutathione peroxidase (GPx). These enzymes eliminate toxic reduction intermediates of oxygen inside the cell, prevent radical formation and so restrict oxygen toxicity. SOD\(^{139}\) removes \( \cdot\text{O}_2^- \) catalytically by promoting the dismutation of \( \cdot\text{O}_2^- \) to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \); catalase removes \( \text{H}_2\text{O}_2 \) when present in high concentrations; GPx\(^{140}\) removes \( \text{H}_2\text{O}_2 \) when present in low concentrations and can remove organic hydroperoxides by converting reduced glutathione (GSH) to oxidized form (GSSG); and GR substitutes the GSSG for the reaction of GPx.

Non-enzymic antioxidants, ascorbic acid scavenges \( \cdot\text{OH} \) radical and plays a role in the recycling of vitamin E, a membrane antioxidant. Defense mechanisms in membranes include vitamin E, betacarotene and coenzyme Q. Vitamin E (\( \alpha \)-tocopherol) is a very effective, lipid-soluble, chain-breaking antioxidant. Betacarotene is also a lipid-soluble antioxidant and plays a role as a radical scavenger and singlet oxygen quencher. These lipid-soluble antioxidants also have antioxidant effects in lipoproteins. Coenzyme Q is another membrane antioxidant, which also plays a major role in cellular energy metabolism.
2.1.2.5 Medicinal Plants as Antioxidants

Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity. Recently there has been an upsurge of plant antioxidants in reducing such free radical induced tissue injury due to their presumed safety, nutritional and therapeutic values.

Nutraceuticals are supposed to hold the key to a healthy society in the coming future. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body’s ability to use free radicals constructively. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocochromanols) ascorbic acid and carotenoids. Flavonoids are group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action.

The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge since last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. India has a rich history of using various herbs and herbal components for treating various diseases. Employing recent sophisticated techniques many Indian plants have been investigated for their beneficial use as antioxidants or source of antioxidants.

Scartezzini and Speroni have reviewed extensively Curcuma longa Linn. Magnifera indica Linn. Momordica charantia L., Phyllanthus emblica L., Santalum album L., Swertia chirata (Wall.) C. B. Clarke and Withania somnifera (L.) Dunal that posses antioxidant activity which are used in Indian traditional medicine. Recently

Free radical scavenging potential of medicinal plants offers maximum protection to the myocardium in the experimental animals, few such plants are discussed and presented in sequel.

Cardioprotective efficacy of hydro-alcoholic extract of *Ocimum sanctum* L. (Os) (Tulsi) studied by Arya et al. proved the antioxidant potential of Os. Significant ventricular dysfunction, myocardial necrosis and depletion of endogenous antioxidants were observed in the isoproterenol (ISP) treated group compared to sham. Os pre-treatment augmented the basal endogenous antioxidants and restored the antioxidant status of the heart. The myocardial salvaging beneficial effects was translated into functional recovery of the myocardium.

Sharma et al. investigated the cardioprotective potential of hydro-alcoholic extract of *Ocimum sanctum* L. in isoproterenol-induced myocardial infarction in rats. *Ocimum sanctum* (Os) at different doses (25, 50, 75, 100, 200 and 400 mg/kg) was given to animals with isoproterenol induced (200mg/kg) myocardial infarction in rats. Os at the dose of 25, 50, 75 and 100 mg/kg reduced significantly glutathione (GSH), superoxide dismutase (SOD) and LDH levels. It also inhibited the lipid peroxidation as observed by the reduced thiobarbituric acid reactive substances (TBARS) levels. Os at the dose of 50 mg/kg was found to demonstrate maximum cardioprotective effect.
The study designed by Mohanty et al. was to evaluate the cardioprotective potential of hydro-alcoholic extract of *Withania somnifera* (L.) Dunal. A significant decrease in glutathione (p<0.05), activities of superoxide dismutase, catalase (p<0.01) as well as increase in lipid peroxide was observed in the hearts of isoproterenol control group rats as compared to sham control. The data showed that *Withania somnifera* (25, 50, 100mg/kg) exerts a strong cardioprotective effect in the experimental model of isoprenaline-induced myocardial necrosis in rats. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered parameters may contribute to its cardioprotective effect. Among the different doses studied, *Withania somnifera* at 50mg/kg dose produced maximum cardioprotective effect.

Reactive oxygen species (ROS) are implicated in many pathogenic processes including the cardiovascular damages. Detoxification of ROS by antioxidants (AO) therefore affords protection against such diseases. Munasinghe et al. examined the antioxidant potential of nine plants that are components of Ayurvedic formulations used by Sri Lankan traditional medical practitioners for cardioprotection. Aqueous freeze dried extracts of the plants were prepared and the antioxidant activity was measured by both *in vitro* and *in vivo* methods. *Terminalia arjuna* (Roxb.) showed highest antioxidant activity than *Cassia fistula* L. and *Vitex negundo* Linn. Overall results show that only some plants used in the therapy of cardiovascular diseases exert their beneficial effects via antioxidant activity.

The medicinal values of the flowers of *Hibiscus rosa-sinensis* L. have been mentioned in ancient literature as useful in disorders of the heart. This was tested and proved by Gauthaman et al. Dried pulverized flower of *Hibiscus rosa-sinensis* was administered orally to Wistar albino rats in three different doses [125, 250 and 500mg.kg in 2% carboxy methyl cellulose (CMC)], 6 days per week for 4 weeks. The principal finding of the study is that ischemic reperfusion injury (IRI) was associated with oxidative stress, as evidenced by an increase in myocardial TBARS and depletion of the myocardial endogenous antioxidant status (SOD, GSH and Catalase).
Chronic oral administration of *Hibiscus rosa-sinensis* prevented the oxidative stress and the structural changes associated with IRI. The mechanism of such protection of chronic oral administration of *Hibiscus rosa-sinensis* may be due to myocardial adaptation and oxidative stress, which is mediated through augmentation of cellular antioxidants such as glutathione, SOD and catalase.

An antioxidant system has presumably evolved in aerobic organisms\(^{156}\) to protect cells against oxidative damage by oxidants, which are produced during the oxygen metabolism in mitochondria. A mechanism similar to this is observed in *Centella asiatica* (CA)\(^{157}\) mainly due to the presence of phenolic compounds\(^{158}\). Gnanapragasam\(^{96}\) *et al.* evaluated the cardioprotective effect afforded by *Centella asiatica* on mitochondrial antioxidants in ADR induced cardio toxicity. A significant (\(p<0.05\)) decrease in the activities of mitochondrial antioxidant enzymes such as GPx, GST, SOD, CAT and level of GSH were observed in group 2 ADR intoxicated rats. Activities of these enzymes in mitochondria were maintained to near normal (\(p<0.05\)) levels upon CA pre-co-treatment.

In Ayurveda one of the traditional systems of Indian medicine, used *Picrorhiza kurroa*, a medicinal plant, to cure cardiac ailments\(^{97}\). Picroside I, Picroside II and Kutkoside are the naturally occurring free radical scavenging principles present in the roots and rhizomes of *P. Kurroa*\(^{159, 160}\). Rajaprabhu\(^{97}\) *et al.* have examined the protective effect of ethanolic extract of *P. kurroa* in adriamycin-induced cardiomyopathy in rats with respect to changes in the levels of lipid peroxidation and antioxidant status. Intraperitoneal administration of adriamycin caused significant (\(p<0.05\)) elevation in the level of lipid peroxidation in the heart tissue ADR administered rats as compared to that of normal rats. This was paralleled by a decline in the level of reduced glutathione and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes. Oral pretreatment with PK significantly (\(p<0.05\)) counteracted all these adriamycin-induced adverse effects and maintained the myocardial antioxidant defense system at a status comparable to that of control animals.
Many indigenous herbal plants of regional interest have been used popularly as folk medicines in Taiwan or other Asian countries; however, their bioactivities or pharmacological effects remained to be elucidated. In this study, Shyur et al. investigated 26 selected, local putative medicinal plants for their potential antioxidant activities using free radical scavenging activity assays. The results demonstrated that, among the tested plant extracts, *Ludwigia octovalvis* (Jacq.) P.H. Raven and *Vitis thunbergii var. taiwaniana* extracts exhibited most potent antioxidant activities.

There are several Indian medicinal plants known for their beneficial therapeutic effects, which also might be due to their antioxidant properties. *Terminalia arjuna* is one of these plants credited for its cardiotonic and cardioprotective properties. The bark of the tree is used in ‘Ayurvedic’ system of medicines for over three centuries, primarily as cardiac tonic besides being used in haemorrhages, fractures, diarrhoea, ulcers and acne. The radical scavenging activities and membrane protective abilities of *T. arjuna* extracts, as well as baicalein, its active ingredient, were examined by Tilak et al. The results have shown that methanolic and aqueous extracts of *T. arjuna* as well as baicalein, when present during radiation exposure, can prevent the damage to the cell membranes by scavenging free radicals. *T. arjuna*’s active constituents include tannins, triterpenoid saponins, gallic acid, ellagic acid, oligomeric proanthocyanidines, phytosterols, flavonoids such as arjunone, arjunolone, luteolin, baicalein etc. besides inorganic constituents such as Ca, Mg, Zn, Cu were also present.

Specific effects of betaine on cellular function have been reported to include reduced hepatic lipidosis and necrosis, improved morphology of mitochondria, rough endoplasmic reticulum, Golgi complexes, and nuclear DNA and increased S-adenosyl methionine content. Studies have shown that betaine exerts cellular and subcellular membrane stabilization in the liver by restoring both enzymatic and non-enzymatic antioxidants. Injection of isoprenaline induced significant (p<0.001) elevation in the level of lipid peroxides in the heart mitochondria of Isoprenaline treated rats as compared with that of control animals. A Parallel decline in the level of GSH and in
the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes was observed. Prior oral administration of betaine significantly (p<0.001) prevented all these isoprenaline-induced adverse effects and maintained the heart mitochondrial antioxidant defense system at near normal. Thus Ganesan et al. clearly indicated the effects of betaine in maintaining antioxidant defense system.

Natural antioxidants from fruits and vegetables provide a measure of protection that slows down the process of oxidative damage. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables. Fruits and vegetables are high in flavonoid content; it is estimated that humans consume between few hundred milligrams and one gram of flavonoids every day. Human studies have shown that flavonoids appear in blood plasma, at pharmacologically active levels, after eating certain foods but do not accumulate in the plasma. Certain flavonoids are excreted in urine within 4 h of ingestion. Regular consumption of flavonoids may increase longevity by reducing inflammation and contributing to a reduction in CHD.

Sangeetha and Darlin Quine evaluated the preventive effect of S-allyl cysteine sulfoxide (SACS) on lipid peroxidative products and enzymic and non-enzymic antioxidants in isoproterenol induced myocardial infarction in rats. The concentrations of thiobarbituric acid reactive substances and lipid hydroperoxides were increased in hearts from ISO-treated rats, whereas the content of enzymic and non-enzymic antioxidants were declined in ISO administered rats. Oral pretreatment with SACS significantly (p<0.05) increased antioxidants in ISO-induced rats.

One of the most important characteristics of glutamine is that it plays a critical role in glutathione biosynthesis by providing glutamate to the glutathione system, which is one of the main sources of the antioxidant defense system in the cell. It is also involved in cell membrane stabilization, antioxidation, detoxification, and energy production. Significant reduction in the intracellular concentration of glutamine has been reported to occur in the heart tissue during myocardial infarction. Kumar
and Anandan\textsuperscript{111} observed a significant (p<0.001) increase in the level of lipid peroxidation along with a concomitant decline in the level of GSH noted in the heart tissue of isoprenaline-administered rats as compared to controls. Also a significant (p<0.001) reduction in the activities of glutathione dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) were observed. The prior administration of glutamine significantly reduced the isoprenaline induced adverse effects and maintained the level of evaluated parameters at near normalcy. Oral administration of glutamine resulted in a significant (p<0.01) elevation in the level of reduced glutathione in experimental animals.

Silymarin is a mixture of three bioflavonoids silybin, silydianin and silychristin isolated from the milk thistle plant \textit{Silybum marianum} (L.) Gaertn\textsuperscript{177}. Silybin is the main component (60\% to 70\%) and is believed to exhibit rich most biological activity. Silymarin can prevent lipid peroxidation\textsuperscript{178-181}, scavenge reactive oxygen species\textsuperscript{182-184}, increase antioxidative enzyme levels \textsuperscript{185, 186} and limit lipid peroxidation\textsuperscript{186}. Rao\textsuperscript{116} et al. proved the cardioprotective activity of Silymarin by evaluating the levels of antioxidants. In the ischemia reperfusion control group, the endogenous antioxidant enzymes such as SOD, CAT, GST and GSH levels were depleted in heart tissue as compared with the sham control group. In rats pretreated with silymarin the antioxidant enzymes levels in heart tissue were brought back to near normal and the difference was also significant (P<0.05) as compared with the control group.

Dhandapani\textsuperscript{187} et al. studied the cardioprotective efficacy of squalene, an isoprenoid antioxidant molecule. Intraperitoneal injection of isoprenaline caused a significant rise in the level of lipid peroxidation with concomitant decline in the level of reduced glutathione (GSH) and in the activities of glutathione-dependent antioxidant enzymes; glutathione peroxidase (GPx) and glutathione-S-transferase (GST), and antiperoxidative enzymes; superoxide dismutase (SOD) and catalase (CAT) in heart tissue. Combined supplementation of squalene and PUFA concentrate significantly prevented the isoprenaline-induced elevations in the levels of diagnostic
marker enzymes in plasma of experimental rat model. A tendency to counteract the isoprenaline induced lipid peroxidation was also noticed. Their combined administration maintained the level of GSH and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes at near normalcy.

Curcumin, the main yellow bioactive component of turmeric (*Curcuma longa* L., Zingiberaceae), a potent antioxidant act as a scavenger of oxygen free radicals. Nazam Anzari et al. proved the protective role of curcumin in myocardial oxidative damage induced by isoproterenol in rats. Myocardial LPO was found to be significantly (P < 0.01) higher in the pathogenic control group. Treatment with curcumin significantly (P < 0.01) decreased the elevated level of LPO. The levels of myocardial endogenous antioxidants (SOD, CAT, and TG) decreased significantly (P < 0.01) in the pathogenic control group as compared with the vehicle control group, and the levels of myocardial endogenous antioxidants viz. SOD, CAT, and TG increased significantly (P < 0.01) with curcumin treatment, as compared with the pathogenic control group.

Ursolic acid (UA; 3-hydroxy-urs-12-en-28-oic acid), a steroid-like triterpene compound is present in many kinds of medicinal plants, such as *Eriobotrya japonica* (Thunb.) Lindl., *Rosmarinus officinalis* L., *Melaleuca leucadendron* auct. non(L.) L., *Ocimum sanctum* Linn, *Glechoma hederaceae* L. and *Piper betle* L. in the form of free acid or as aglycone of triterpenoid saponins. Saravanan et al. studied the impact of ursolic acid on chronic ethanol-induced oxidative stress in the rat heart. Chronic ethanol administration significantly enhanced the myocardial lipid peroxidation as compared to normal control. The levels of lipid peroxidation markers such as TBARS, LOOH and CD were maintained at near normal levels in rats co-administered with UA. Ursolic acid exhibited its potent antioxidant activity by decreasing the levels of TBARS and CD in control rats administered with UA as compared to normal control rats. The activities of SOD, CAT, GPx and GST substantially decreased in the heart of ethanol-administered group. In response to UA...
treatment, the activities of these enzymic antioxidants increased significantly ($p < 0.05$) as compared to untreated alcoholic rats.

Protective role of curcumin against isoproterenol-induced myocardial infarction in rats was evaluated and proved by Nirmala and Puvanakrishnan$^{193}$. Myocardial infarction was accompanied by the disintegration of membrane polyunsaturated fatty acids as expressed by increase of thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxides and by the impairment of natural scavenging, characterized by the decrease in the levels of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin, alpha tocopherol, reduced glutathione (GSH) and ascorbic acid. The oral pretreatment with curcumin, two days before and during ISO administration decreased the effect of lipid peroxidation. It was shown to have a membrane stabilizing action by inhibiting the release of β-glucuronidase from nuclei, mitochondria, lysosome and microsome.

Rajadurai and Prince$^{194}$ designed a study to evaluate the cardioprotective potential of naringin on lipid peroxides; enzymatic and nonenzymatic antioxidants in ISO induced myocardial infarction in rats. Subcutaneous injection of ISO (85mg/kg) showed a significant increase in the levels of thiobarbituric acid reactive substances and lipid peroxides in plasma and the heart and a significant decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the heart and the levels of glutathione, vitamin C and Vitamin E in plasma and heart and ceruloplasmin in plasma. Oral administration of naringin (10, 20, and 40mg/kg) to ISO-induced rats daily for a period of 56 days showed a significant decrease in the levels of lipid peroxidative products and improved the antioxidant status by increasing the activities of enzymatic and nonenzymatic antioxidants.

‘Abana’, is a cardiotonic formulation of selected ingredients$^{195}$, which provides significant protection against ischemia$^{196}$ and hypertension$^{197}$. It is a cardioprotective drug with antithrombotic and antihypercholestaemic effects$^{198}$. 

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Sasikumar and Devi\(^{199}\) studied the effect of ‘Abana’ on lipid peroxidation in experimental myocardial infarction in rats. There was a significant increase in lipid peroxides levels on isoproterenol administration. Activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase, glutathione reductase were decreased significantly in heart with isoproterenol-induced myocardial necrosis. ‘Abana’, produced a marked reversal of these metabolic changes related to myocardial infarction induced by isoproterenol.

A.V. Circulo (AVC) is a polyherbal formulation containing the most well documented Asian herbs for protecting the heart and improving heart function viz., Terminalia arjuna (Roxb.), Crataegus oxycantha L., Withania somnifera (L.) Dunal, Boerhaavia diffusa L., Coleus forskohlii, and Piper longum Briq. These herbs are known to improve blood flow to the heart and stabilize the rate of heart contraction and blood pressure, when taken regularly\(^{200-202}\). The phyto-constituents of AVC are documented to possess potent cardiotonic and free radical scavenging activity\(^{203,204}\).

Naik and Panda\(^{118}\) observed a significant decline in myocardial GSH (p<0.001) in the ISO treated group as compared to the normal group of rats. IAVC21 as well as IAVC45 treatments failed to restore significantly the ISO depleted GSH levels. ISO treatment induced a significant depletion of antioxidant enzymes SOD (p<0.001), CAT (p<0.001), GPx (p<0.001) & GR (p<0.001) in rat hearts. Oral administration of AVC for 21 days as well as 45 days to rats, followed by ISO injection, restored significantly (p < 0.001) the activities of CAT & GPx; whereas, activities of SOD and GR were significantly (p<0.01) restored only in the IAVC45 group of rats.

### 2.1.3 Lipids, Lipoproteins and Membrane Bound ATPases

Most cardiovascular events are secondary to atherosclerosis, a disease of the arteries involving a local thickening of the vessel wall. A stroke or myocardial infarction occurs when the lumen of the vessel becomes completely occluded, usually by a thrombus forming at the site of a plaque. Atherosclerotic lesions thought to be initiated by emigration of monocytes into the arterial inner core (tunica intima), recruited by adhesion molecules, possibly in response to arterial endothelium.
A variety of factors have been implicated in causing this initial injury, including mechanical damage from flow stress worsened by high blood pressure, viral infection (herpes viruses and cytomegalovirus), exposure to blood-borne toxins such as xenobiotics from cigarette smoke and elevated levels of normal metabolites, such as glucose, homocysteine or cholesterol. Although a high level of plasma cholesterol is considered to trigger atherosclerosis, the oxidation of cholesterol seems to be a necessary step.

Oxidized LDL (oxLDL) acts as a trigger to initiate endothelial inflammation leading to atherosclerosis and vascular thrombosis (heart attack and stroke). oxLDL and oxidized lipoproteins have been reported to stimulate O$_2^-$ formation leading to apoptosis of cells in the umbilical vascular wall; this was prevented by treatment with antioxidants SOD and catalase. Endothelial cells, smooth muscle cells (SMCs), and macrophages are the sources of oxidants for the oxidative modification of phospholipids. In the atherosclerotic lesion produced in the rabbit aorta, significant increase in the iron content was observed suggesting that iron-catalyzed free radical reactions associated with the development of atherosclerosis. Endothelial dysfunction may play an important role in the atherosclerotic process because in patients with atherosclerosis, the antioxidants, probucol and ascorbic acid, improved the endothelium-dependent relaxation suggesting the involvement of ROS in endothelial dysfunction. Increased production of O$_2^-$ implicated in the impaired endothelium-dependent relaxation was suggested to be an early event in the hypercholesterolemic atherosclerotic process.

Oxidative inactivation of NO$^+$ by superoxide has been proposed as a plausible explanation for endothelial dysfunction. When exposed together, O$_2^-$ and NO$^+$ react with each other three times faster than the reaction rate of O$_2^-$ with either Mn$^{2+}$ or Cu$^{2+}$/Zn$^{2+}$-SOD. Therefore, O$_2^-$ would preferentially react with NO$^+$ rather than SOD. In human atherosclerotic arteries, the production of endothelial nitric oxide synthase (the enzyme catalyzing NO$^+$ formation) as well as NO$^+$ has been shown to be depressed. The generation of O$_2^-$ was thought to be due to the activation of the

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vascular and endothelial enzyme NADH/NADPH oxidase\textsuperscript{214}. Moreover, an increase in NADH/NADPH oxidase-dependent vascular $O_2^-$ was reported in hypercholesterolemic rabbits\textsuperscript{215}. Oxidation of NO' by $O_2^-$ results in the formation of peroxynitrite, which could initiate lipid peroxidation or play a role in the oxidation of lipoproteins\textsuperscript{216, 217}.

\textit{Figure 6: Development of Atherosclerosis.} [Adapted from: Madamanchi NR, Vendrov A, Runge MS. Oxidative Stress and Vascular Disease. \textit{Arterioscler Thromb Vasc Biol} 2005; 25: 29-38]

The basis for life is the ability of the cell to maintain ion gradients across biological membranes. Specific membrane-bound ion pumps [adenosine triphosphatases (ATPases)] created such gradients\textsuperscript{218}. ATPases are membrane bound enzymatic proteins that maintain ionic gradients between aqueous intra and extra cellular phases\textsuperscript{219}. Membrane bound enzymes such as Na\textsuperscript{+}/K\textsuperscript{+} ATPase, Mg\textsuperscript{2+} ATPase and Ca\textsuperscript{2+} ATPases are responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis\textsuperscript{220}. Alterations in the activities of these enzymes are due to increased production of free
radicals leading to cell injury. Free radicals have been suggested to exert their cytotoxic effects by causing peroxidation of membrane lipids.  

Damage of plasma membrane occurs directly through interaction with the membrane components such as the ion-dependant ATPases and ion channels and indirectly as a consequence of overt cytosolic damage. Inhibiting function of ion-dependant ATPases leads to disturbances in ion homeostasis resulting in impaired signal transduction, altered cellular metabolism, changes in cell membrane permeability and integrity, elevation in membrane fluidity and disturbances of vital functions. Ca\textsuperscript{2+}ATPase is also located in the plasma membrane, pumping Ca\textsuperscript{2+} out of the cell thereby helping to maintain the concentration gradient of Ca\textsuperscript{2+} between the cytosol and the extra cellular fluid. Many ATPases, including Ca\textsuperscript{2+}ATPase, contain essential sulfhydryl groups. Impairment of this enzyme may be due to peroxidative stress, which may act on the sulfhydryl groups present in the active site of Ca\textsuperscript{2+}ATPase. Thiol modification (i.e. loss of protein sulfhydryl groups) has been recognized as critical event in cytotoxicity. Damage to these thiol moieties may result in the inhibition of Ca\textsuperscript{2+}ATPases, function and increase in the intracellular Ca\textsuperscript{2+} concentration may result.

Kumar et al. investigated the cardioprotective effect of the ethanol extract of Picrorhiza kurroa Royle ex Benth rhizomes and roots (PK) on isoproterenol-induced myocardial infarction in rats with respect to lipid metabolism in serum and heart. Isoproterenol treated rats showed a significant increase in the levels of total cholesterol, triglycerides, phospholipids, LDL and VLDL with a significant decrease in HDL in serum. There was also a significant increase (p<0.05) in the levels of total cholesterol, triglycerides, phospholipids and free fatty acids, and a decrease in the concentration of phospholipids was observed in the heart of ISO-treated rats. Oral pretreatment with PK (80mg/kg/day for 15 days) significantly prevented the isoproterenol-induced myocardial infarction and maintained the rats at near normal status.

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Literature Review

*Aegle marmelos* (L.) Corr. Serr, commonly known as Bael, a cardiotonic and is one of the most useful medicinal plants in India. Rajadurai and Prince\(^{226}\) studied the comparative effects of *Aegle marmelos* leaf extract (AMLEt) and alphatocopherol on plasma lipids, lipid peroxides and marker enzymes in rats with isoproterenol (ISO)-induced myocardial infarction. Increased levels of total, free and ester cholesterol, triglycerides, phospholipids and free fatty acids in serum of rats treated with ISO were also observed. An increase in serum LDL and VLDL fractions, along with a decrease in HDL cholesterol, was also observed in ISO-treated rats. Pre-treatment with AMLEt at doses of 100 mg/kg and 200 mg/kg body weight for 35 days showed a significant effect on lipids, lipoproteins and antioxidant enzymes in ISO-treated rats. The effect of AMLEt 200 mg/kg was found to be almost similar to that of alphatocopherol 60 mg/kg.

Hypertension and dyslipedemia are major risk factors of cardiovascular morbidity and mortality and a continuing challenge to public health efforts. In India, different medicinal systems make use of a number of plants in the treatment of hypertension. Raja\(^{227}\) *et al.* studied the effect of *Melothria maderaspatana* (Linn.) leaf-tea consumption on lipid profile in patients with hypertension. The total cholesterol, LDL, triglycerides and phospholipids levels decreased significantly and HDL and serum bilirubin levels increased after tea consumption in patients with hypertension.

The bark of *Terminalia arjuna* (family Combretaceae) is an Ayurvedic remedy that has been mentioned in many books on Indian Meteria Medica such as Charaka Samhita and Astang Hridayam, as to possess cardio protective Property\(^{228}\). A number of experimental and clinical studies have proved that dried bark powder of this plant have potent hypolipidemic and cardioprotective activity\(^{229-232}\). Antidyslipidemic and antioxidant activities of different fractions of *Terminalia arjuna* stem bark were studied by Chander\(^{233}\) *et al.* Treatment of hyperlipidemic rats with *T. arjuna* fractions A, B, C and D at the dose of 250 mg/Kg p.o. reversed the plasma levels of lipid but with varying extents.

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*Cardioprotective Effect of Muntingia calabura L.* - A Traditional Drug Source
**Daucus carota** Linn. (Family: Umbeliferae) is an annual or biennial herb, whose roots are eaten raw and also cooked in many parts of the world. The root of the plant is used as a diuretic and inotropic\(^{234}\). Muralidharan\(^{101}\) et al. estimated the cardiac tonicity by evaluating Na\(^+\)K\(^+\)ATPase, Mg\(^+\)ATPase and Ca\(^+\)ATPase in heart. The levels of Na\(^+\)K\(^+\)ATPase and Mg\(^+\)ATPase decreased and that of Ca\(^+\)ATPase increased in extract (aqueous) -treated group significantly (p<0.001) as compared to isoproterenol treated animals.

Elevated serum cholesterol (and hence LDL cholesterol) has consistently been shown to be a significantly risk factor for coronary heart disease (CHD) and other major CVD as well \(^{235}\). Any damage to the tissues as a result of lipid accumulation or fatty infiltration could result in a significant increase in serum values becoming a valuable diagnostic tool for cardiovascular disease. Oluba\(^{236}\) et al. studied the comparative effect of soybean oil and palm oil on serum lipids in cholesterol-fed rats. The animals consumed the daily rations satisfactorily and grew well during the study. At the end of the feeding period, rats fed with cholesterol diet enriched with palm oil had significantly higher (p<0.05) body weight compared to those fed cholesterol diet supplemented with soybean oil. However, the control rats showed significantly lower growth response compared to both palm oil- and soybean oil-fed rats. From the results it is observed that the palm oil fed rats and the soybean fed rats had significantly lower serum cholesterol compared with the control, these decreases were even lower in the palm oil fed group as compared with the soybean oil group. Serum triglyceride concentrations were lowest in the palm oil- fed rats followed by the soybean fed group. The control rats showed a significantly higher serum triglyceride values compared to both groups. In conclusion, the data generated in this study clearly showed that palm oil consumption had better protection against coronary heart disease risk than soybean oil.

Intraperitoneal administration of isoprenaline caused fatty changes in the myocardium. Hyperlipidemia is one of the major factors responsible for the occurrence of myocardial infarction\(^{237}\). Injection of isoprenaline to rats increased the
low-density lipoprotein (LDL) cholesterol level in the blood, which in turn lead to the build-up of harmful deposits in the arteries, and thus favored myocardial infarction. The action of squalene on isoprenaline-induced changes in lipid profiles in plasma and heart tissue in male albino rats was studied by Farvin et al. Squalene exerted an antilipidemic effect by reducing the level of low-density lipoprotein cholesterol with a parallel rise in the level of high-density lipoprotein cholesterol in the plasma of experimental rats. A tendency to prevent the isoprenaline-induced depletion of phospholipids in the myocardium of experimental rats was also observed. The results of the present study indicated that the overall cardioprotective effect of squalene is probably related to an inhibition of lipid accumulation by its hypolipidemic properties.

Oleanolic acid (OA) is a triterpenoid compound that exists widely in food and herbs. It has a variety of biological effects, such as anti-oxidants, anti-hyperlipidemia, hepatoprotective, tumor prevention, immunomodulatory, anti-HIV, anti-arrhythmic and cardiotonic. Senthil et al. designed a study to investigate the protective effect of oleanolic acid (OA); against isoproterenol-induced myocardial ischemia in rat myocardium with respect to lipids, lipoproteins and membrane bound ATPases. Increased lipid profiles and decreased activities of membrane-bound ATPase enzymes confirmed the severe myocardial damage occurring as a consequence of isoproterenol-induced ischemia, and they also observed a significant improvement by oleanolic acid pretreatment.

Chitosan, a marine polysaccharide was examined for its antilipidemic potential by Sivakumar et al. on isoprenaline-induced myocardial infarction. Injection of isoprenaline into rats increased the low-density lipoproteins (LDL) cholesterol level in the blood, which in turn lead to the build up of harmful deposits in the arteries, and thus favored myocardial infarction. It exerted significant antilipidemic effect against isoprenaline-induced myocardial infarction by maintaining the levels of cholesterol, triglycerides, free fatty acids and phospholipids in plasma and heart tissue as compared to that of control animals. The overall...
cardioprotective effect of chitosan is probably related to its ability to inhibit the isoprenaline-induced lipid accumulation by its hypolipidemic property.

Gum guggul, which is obtained from the herb Commiphora mukul, was recommended in the treatment of atherosclerosis\textsuperscript{244}. Guggulipid is at present commercially manufactured in India, and it seems to be the first hypolipidemic agent derived from the plant source\textsuperscript{245}. Singh\textsuperscript{246} et al. conducted a study to investigate hypolipidemic and antioxidant effects of Commiphora mukul as an adjunct to dietary therapy in patients with hypercholesterolemia. The effects of the administration of 50 mg of guggulipid was studied by giving in capsule form, twice daily for 24 weeks to 31 in the guggulipid group and 30 in the placebo group and a fruit- and vegetable-enriched prudent diet to all 61 patients with hypercholesterolemia in a randomized, double blind fashion. Guggulipid decreased the total cholesterol level by 11.7%, LDL by 12.5%, triglycerides by 12.0%, and the total cholesterol/HDL cholesterol ratio by 11.1% from the post diet levels, whereas the levels were unchanged in the placebo group. Thus guggulipid was proved to be a good hypolipidemic agent.

Ursolic acid is present in many plants, including apples, basil, bilberries, cranberries, elder flower, peppermint, rosemary, lavender, oregano, thyme, hawthorn, prunes. Senthil\textsuperscript{107} et al. studied the protective effects of ursolic acid (UA) against myocardial ischemia with reference to lipid profile and membrane bound enzymes. Isoproterenol administration significantly increased the lipid profile (total cholesterol, free cholesterol, ester cholesterol, triglycerides, free fatty acids and phospholipids) and significantly decreased the activities of membrane bound enzymes (Na$^+$K$^+$ATPase, Mg$^{++}$ATPase and Ca$^{++}$ATPase). The prior administration of UA significantly prevented the Isoproterenol-induced alterations in lipid profile and restored the enzymes to near normal.

Prince\textsuperscript{247} et al. studied the preventive effect of rutin on lipids, lipoproteins and ATPases in normal and isoproterenol-induced myocardial infarction in rats. The concentration of total cholesterol, triglycerides and free fatty acids increased...
significantly and the concentration of phospholipids decreased significantly on isoproterenol (150mg/kg) administration in the heart of experimental animals. In serum also, a significant increase was observed (p<0.05) in the levels of total cholesterol, triglycerides, phospholipids, low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) with a significant decrease in high-density lipoprotein cholesterol (HDL-C). The activities of sodium potassium dependent adenosine triphosphatase (Na\(^+\)/K\(^+\) ATPase) and magnesium dependent adenosine triphosphatase (Mg\(^2+\) ATPase) also decreased significantly (p<0.05), and the activity of calcium dependent adenosine triphosphatase (Ca\(^2+\) ATPase) increased significantly in the heart of ISO-treated rats.

Hyperlipidemia is one of the risk factors for coronary artery disease. In unani pharmacopoeia several formulations have been described for the treatment of cardiovascular diseases. Tajuddin et al. evaluated the role of a formulation containing aqueous extracts of *Bombyx mori* L., *Nepeta hindostana* (Roth) Haines and *Terminalia arjuna* (Roxb.) in the ratio of 1:2:1 respectively in hyperlipidemia. Isoproterenol treated group showed a significant increase in total cholesterol, triglycerides, phospholipids, free fatty acids, VLDL and LDL as compared to the normal control and the levels were significantly brought back to near normal status in unani formulation pretreated rats. Serum high-density lipoprotein (HDL) was lowest in isoproterenol treated groups as compared with control and unani formulation pretreated group.

### 2.1.4 Mitochondrial, Lysosomal and Glucose metabolizing enzymes

Mitochondria are the important places for the metabolism of energy in cells, in which sugar, fat and amino acids are eventually required to be oxidized. Energy released through the process of oxidation is stored in the high-energy bond of adenosine triphosphate. It is the effect of oxidative phosphorylation that provides the main energy for living. Studies on injury of myocardium that resulted from hypoxia, changes the structure and oxidative phosphorylated function in mitochondria.
Oxidative phosphorylation is the process by which ATP is formed as electrons transferred from NADH or FADH₂ (generated through the Krebs cycle) to molecular oxygen. This occurs through a series of electron transport carriers localized in the inner mitochondrial membrane. The electron transport carriers include: complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (ubiquinol-cytochrome c reductase), and complex IV (cytochrome c oxidase) (Figure 7). The transfer of more than 98% of electrons by the electron transport carriers/chain is coupled with the production of ATP. Only 1% to 2% of electrons leak out to form O₂, and this is scavenged by manganese SOD (MnSOD/SOD2). However, during mitochondrial oxidative phosphorylation under pathophysiological conditions, the electron transport chain may become uncoupled, leading to increased •O₂⁻ production.³⁴⁹

Figure 7: •O₂⁻ Production in Mitochondria. [Adapted from: Madamanchi NR, Vendrov A, Runge MS. Oxidative Stress and Vascular Disease. Arterioscler Thromb Vasc Biol 2005; 25: 29-38]
Lysosomal enzymes play an important and well-established role in the breakdown of necrotic cells\(^{250,251}\). These enzymes are mainly acid hydrolases stored in subcellular organelles (lysosomes). Membrane damage is due to high oxygen demand, which leads to hypoxic state. Hypoxia affects oxidative phosphorylation in mitochondria and this causes the available ATP levels in the cells to decline. With the decrease of ATP, cellular AMP level increases. This increases AMP: ATP ratio and stimulates phospho fructo kinase (PFK – 1) enzyme. This enhances the rate of anaerobic glycolysis. Increased glycolysis leads to the depletion of glycogen stores and an increase in intracellular lactic acid levels. This increases intracellular acidity (decrease in pH). Falling of the intracellular pH destabilizes the lysosomal membrane and hydrolytic enzymes in the lysosomes come out. These enzymes hydrolyze intracellular biological molecules and ultimately lead to cell death.

Among the sequel of hyperglycemia, excess oxidative stress has captured considerable attention as a potential mechanism for the increased vascular disease in diabetics. The established association between atherosclerosis and lipid peroxidation in plasma\(^ {252}\) and within the vascular wall\(^ {253}\) has led to a renewed interest in the oxidative stress of hyperglycemia as a potential mechanism for diabetic vascular disease. There are a number of other putative mechanisms that link hyperglycemia to oxidative stress. Among the most direct mechanism is the autooxidation of glucose. Monosaccharides with a \(\alpha\)-hydroxyaldehyde structure, like glucose, are subjected to enediol rearrangement that results in the formation of an enediol radical ion\(^ {254}\). The formation of this radical anion has two important implications. First, this species is capable of reduced molecular oxygen to form superoxide anion, which, under certain circumstances, may contribute to the oxidation of lipids\(^ {255}\) or the activation of platelets\(^ {256}\).

NADH oxidase has been proposed to be a major source of superoxide anion (\(O_2^-\)) in normal and diseased blood vessels\(^ {257}\). The mechanism of impaired endothelium dependent relaxation in diabetes may involve inactivation of nitric oxide.
(NO) by oxygen-derived free radicals \(^{258, 259}\) increased activity of NAD/NADH oxidase in diabetic vessel could play a pivotal role in the early stage of vascular complications in diabetes.

Many plants used to prevent the mitochondrial and lysosomal damage, thereby limiting the myocardial injury are reviewed and presented. Generation of reactive oxygen species and mitochondrial dysfunction has been implicated in adriamycin induced cardiotoxicity. Gnanapragasam \(^90\) et al. aimed to evaluate the efficacy of *Centella asiatica* on the mitochondrial enzymes and mitochondrial antioxidant status in adriamycin induced myocardial injury. Adriamycin (2.5 mg/kg body wt., i.p.) induced mitochondrial damage in rats was assessed in terms of decreased activities \((p < 0.05)\) of TCA cycle enzymes (isocitrate dehydrogenase, \(\alpha\)-ketoglutarate dehydrogenase, malate dehydrogenase) respiratory marker enzymes (NADH-dehydrogenase, cytochrome-C-oxidase), mitochondrial antioxidant enzymes (GPx, GSH, SOD, CAT) and increased \((p < 0.05)\) level of lipid peroxidation. Pre-co-treatment with aqueous extract of *Centella asiatica* (200 mg/kg body wt, oral) effectively counteracted the alterations in mitochondrial enzymes and mitochondrial defense system.

*Nardostachys jatamansi* Jones DC contains various sesquiterpenes, lignans and neolignans in the roots of the plant \(^{260}\). In the Unani system of medicine, *N. jatamansi* has been mentioned as a hepatotonic, cardiotonic, diuretic and analgesic \(^{261}\). Subhashini \(^{262}\) et al. proved the protective efficacy of *Nardostachys jatamansi* (rhizomes) on lysosomal hydrolases during doxorubicin induced myocardial injury. Doxorubicin given rats showed significant changes in the lysosomal enzymes (\(\alpha\)-D-glucuronidase, \(\alpha\)-D-N-acetylglucosaminidase, cathepsin-D, acid phosphatases and \(\alpha\)-D-galactosidase). Myocardial damage was assessed by ultrastructural changes, which revealed loss of myofibrils, mitochondrial swelling and cytoplasmic vacuolization. Pretreatment with *Nardostachys jatamansi* for seven days ameliorated the observed abnormalities and significantly prevented the lysosomal integrity in doxorubicin induced rats.

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**Cardioprotective Effect of Muntingia calabura L. - A Traditional Drug Source**
Suchalatha and Devi\textsuperscript{263} studied the protective effect of \textit{Terminalia chebula} against lysosomal enzyme alterations in isoproterenol-induced cardiac damage in rats. Isoproterenol administration produced significant cardiac damage (as seen by the triphenyltetrazolium chloride assay) and significantly altered lysosomal enzyme activities. Pretreatment with an ethanol extract of \textit{T. chebula} was found to retain near normal activities of lysosomal enzymes in rats as compared with rats given isoproterenol alone.

Karthikeyan\textsuperscript{264} et al. designed a study to examine the effects of grape seed proanthocyanidins (GSP) against myocardial injury (MI) induced by isoproterenol (ISO), in a rat model. Induction of rats with ISO (85 mg/kg body weight, subcutaneously) for 2 days resulted in a significant decrease in the activities of heart mitochondrial enzymes (isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and \(\alpha\)-ketoglutarate dehydrogenase) and respiratory chain enzymes (NADH dehydrogenase and cytochrome C oxidase). The activities of lysosomal enzymes (\(\alpha\)-D-glucuronidase, \(\alpha\)-D-N-acetylglucosaminidase, cathepsin-D, acid phosphatases and \(\alpha\)-D-galactosidase) were increased significantly in the heart and serum of ISO-induced rats. The prior administration of GSP for 6 days a week for 5 weeks significantly increased the activities of mitochondrial and respiratory chain enzymes and significantly decreased the activities of lysosomal enzymes in the heart tissues of ISO-induced rats, which proved the stress stabilizing action of GSP.

Padmanaban and Prince\textsuperscript{265} aimed to evaluate the preventive role of \(S\)-allylcysteine (SAC) on mitochondrial and lysosomal enzymes in isoproterenol (ISO)-induced rats. Male albino Wistar rats were pretreated with SAC (50, 100 and 150 mg/kg) daily for a period of 45 days. After the treatment period, ISO (150 mg/kg) was subcutaneously injected to rats at an interval of 24 h for two days. The activities of heart mitochondrial enzymes (isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and \(\alpha\)-ketoglutarate dehydrogenase) and respiratory chain enzymes (NADH dehydrogenase and cytochrome C oxidase) were decreased significantly \((p < 0.05)\) in ISO-induced rats. The activities of lysosomal enzymes (\(\beta\)-
glucuronidase, β-N-acetyl glucosaminidase, β-galactosidase, cathepsin-D and acid phosphatase) were increased significantly \((p < 0.05)\) in serum and heart of ISO-induced rats. Pretreatment with SAC (100 mg/kg and 150 mg/kg) for a period of 45 days increased significantly \((p < 0.05)\) the activities of mitochondrial and respiratory chain enzymes and decreased the activities of lysosomal enzymes significantly \((p < 0.05)\) in ISO-induced rats.

Intake of dietary flavonoids has been reported as inversely related to the incidence of cardiovascular diseases (CVD). Rajadurai and Prince\(^{266}\) have undertaken a study to evaluate the preventive role of naringin on mitochondrial enzymes in isoproterenol (ISO)-induced myocardial infarction in male albino Wistar rats. Rats subcutaneously injected with ISO (85 mg/kg) at an interval of 24 h for 2 days, resulted in significant \((p < 0.05)\) increase in the levels of mitochondrial lipid peroxides. ISO-induction also showed significant \((p < 0.05)\) decrease in the activities of mitochondrial tricarboxylic acid cycle enzymes (isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and alpha-ketoglutarate dehydrogenase) and respiratory chain enzymes (NADH dehydrogenase and cytochrome c oxidase). Oral pretreatment with naringin (10, 20, and 40 mg/kg) to ISO-induced rats daily for a period of 56 days significantly \((p < 0.05)\) minimized the alterations in all the biochemical parameters and restored the normal mitochondrial function.

Devika and Prince\(^{267}\) aimed to evaluate the preventive role of (-)epigallocatechin-gallate (EGCG) on lysosomal enzymes in isoproterenol (ISO)-induced myocardial infarcted rats. Male albino Wistar rats were pretreated with EGCG (30 mg/kg) daily for a period of 21 days. After the treatment period, ISO (100 mg/kg) was subcutaneously injected to rats at intervals of 24h for 2 days. The activities of lysosomal enzymes (beta-glucuronidase, beta-N-acetylglucosaminidase, beta-galactosidase, cathepsin-B and cathepsin-D) were increased significantly \((P<0.05)\) in serum and the heart of ISO-induced rats. ISO-induction also resulted in decreased stability of membranes, which was reflected by decreased activities of beta-
glucuronidase and cathepsin-D in mitochondrial, nuclear, lysosomal and microsomal fractions. Pretreatment with EGCG daily for a period of 21 days to ISO-induced rats prevented the changes in the activities of these enzymes.

The synergistic protective effect of nicorandil (K (ATP) channel opener) and amlodipine (calcium channel blocker) on mitochondrial respiration and mitochondrial lipid contents and lysosomal hydrolases in serum and heart were examined on isoproterenol-induced myocardial infarction in rats. The rats given isoproterenol (150 mg kg(-1) daily, i.p.) for 2 days showed significant changes in mitochondrial enzymes and increased activities of beta-glucuronidase, beta-N-acetyl glucosaminidase, beta-galactosidase, cathepsin-D and acid phosphatase in serum and heart were also observed. Pretreatment with nicorandil (2.5 mg kg(-1) daily, p.o.) and amlodipine (5.0 mg kg(-1) daily, p.o.) for 3 days significantly prevented these alterations and restored the activities of mitochondrial and lysosomal enzymes to near normal.

Ebenezer et al. studied the protective effect of L-arginine and L-lysine on lysosomal enzymes and membrane bound ATPases was examined on isoproterenol induced myocardial infarction in rats. The rats given isoproterenol (150 mg kg(-1) daily) intraperitoneally for 2 days showed significant changes in the marker enzymes, lysosomal enzymes and membrane bound phosphatases. Prior oral treatment with L-arginine (250 mg/kg daily) and L-lysine (5 mg/kg daily) for 5 days significantly prevented these alterations and restored the enzyme activities to near normal. These findings demonstrated the protective effect of L-arginine and L-lysine in combination against isoproterenol induced cardiac damage.

Hyperglycemia is a risk factor for increased mortality after a myocardial infarction. Whether this applies for hyperglycemic patients with acute myocardial infarction is unclear. Hence the study of glucose metabolizing enzymes is helpful in assessing the severity of hyperglycemia in MI. Reviews on the antidiabetic activity of few plant drugs were discussed in sequel and light is thrown on the effect of the herbs.
in glucose metabolizing enzymes. This will help in understanding the activity of these enzymes during MI and probably may help in understanding MI occurring in hyperglycemia.

Narendhirakannan et al. studied the biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. The study evaluated the hypoglycaemic efficacy of commonly used traditional Indian plants, such as Murraya koenigii L., Mentha piperitae L., Ocimum sanctum and Aegle marmelos, in streptozotocin (STZ)-induced experimental rats. Streptozotocin increased the level of glucose with a concomitant decline in glycogen level and the activities of hexokinase and glucose-6-phosphate dehydrogenase. Oral administration of the ethanolic extract of these plants resulted in a significant decrease in the levels of blood glucose and an increase in the levels of glycogen and enhanced the activities of glucose metabolizing enzymes to near normalcy.

_Eclipta alba_ (L.) Hassk., an indigenous medicinal plant, has a folk (Siddha and Ayurvedha) reputation in rural southern India as a hypoglycemic agent. In order to confirm this claim, Ananthi et al. carried out studies to evaluate the antihyperglycemic effect of _E. alba_ and the activities of liver hexokinase and gluconeogenic enzymes such as glucose-6-phosphatase and fructose 1,6-bisphosphatase in the liver of control and alloxan-diabetic rats. Oral administration of leaf suspension of _E. alba_ (2 and 4 g/kg body weight) for 60 days resulted in significant reduction in blood glucose (from 372.0 ± 33.2 to 117.0 ± 22.8), glycosylated hemoglobin HbAlc, a decrease in the activities of glucose-6 phosphatase and fructose 1, 6-bisphosphatase, and an increase in the activity of liver hexokinase. _E. alba_ at dose of 2 g/kg body weight exhibited better sugar reduction than 4 g/kg body weight.

_Piper longum_ L. is used in Indian traditional medicine as a remedy for various disorders including diabetes mellitus. Manoharan et al. studied the Cardioprotective Effect of Muntingia calabura L.- A Traditional Drug Source
antihyperglycemic effect of ethanolic extract of *Piper longum* (PLEFet) dried fruits in alloxan induced diabetic rats. Diabetes was induced in overnight fasted (12hr) Wistar rats by single intraperitoneal injection of freshly prepared solution of alloxan monohydrate (150mg/kg) in physiological saline. Alloxan administered rats showed an increased glucose level and the activities of glucose-6-phosphatase and fructose 1, 6 bis phosphatase and a parallel decline in the activities of hexokinase and glucose-6-phosphate dehydrogenase. Oral administration of PLEFet has shown antihyperglycemic effect by bringing back the near normal status of altered parameters.

Kumar\(^{274}\) et al. studied the anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. Oral administration of ethanolic extract of the fruits (200mg/kg) for 30 days significantly reduced the alterations in the levels of glucose, insulin and hemoglobin and brought back the near normal activities of glucose metabolizing enzymes.

Sellamuthu\(^{275}\) et al. evaluated the antihyperglycemic potential of mangiferin purified from methanolic root extract of *Salacia chinensis* in control and streptozotocin-induced diabetic animals. The mangiferin was administered orally at a dose of 40mg/kg/day (for 30days) to streptozotocin-induced diabetic rats. The mangeferin treated rats significantly reduced the level of blood glucose, insulin and glycosylated hemoglobin and the activities of glucose-6-phosphatase and fructose 1, 6 bis phosphatase. Mangiferin reversed the activities of hexokinase and glucose-6-phosphate dehydrogenase to near normalcy.

### 2.2 Histopathology and Electrocardiogram

Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides. The tissue is then prepared using histology procedures for viewing under a
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microscope using one of two methods of fixation - chemical fixation or frozen section. As a result of myocardial infarction, occlusion of coronary arteries induced morphological changes in the tissue include various degrees of focal lesions, fragmentation of muscle fiber, which are either reversible or irreversible.

As the heart undergoes depolarization and repolarization, the electrical currents that are generated spread not only within the heart, but also throughout the body. This electrical activity generated by the heart can be measured by an array of electrodes placed on the body surface. The recorded tracing is called an electrocardiogram (ECG, or EKG).

![ECG Tracing](image)

Figure 8: ECG Tracing

The P wave represents the wave of depolarization that spreads from the SA node throughout the atria, and is usually 0.08 to 0.1 seconds (80-100 ms) in duration. The QRS complex represents ventricular depolarization. The duration of the QRS complex is normally 0.06 to 0.1 seconds. The isoelectric period (ST segment) following the QRS is the time at which the entire ventricle is depolarized and roughly corresponds to the plateau phase of the ventricular action potential. The ST segment is important in the diagnosis of ventricular ischemia or hypoxia because under those conditions, the ST segment can become either depressed or elevated. The T wave represents ventricular repolarization and is longer in duration than depolarization (i.e.,...
conduction of the repolarization wave is slower than the wave of depolarization). The Q-T interval represents the time for both ventricular depolarization and repolarization to occur, and therefore roughly estimates the duration of an average ventricular action potential. This interval can range from 0.2 to 0.4 seconds depending upon heart rate. MI exhibited Electrocardiographic (ECG) changes are increased heart rate, reduced R-wave amplitude and ST-segment elevation.

Bestetti et al. studied the ability of the electrocardiogram to detect myocardial lesions in isoproterenol induced rat cardiomyopathy. Resting electrocardiograms were recorded in 18 male adult rats injected subcutaneously with two doses of isoproterenol (200 mg·kg⁻¹ body weight) 10 days before the animals were submitted to the ajmaline test (1 mg·kg⁻¹ body weight i.v). After the ajmaline test all rats were killed and the hearts examined histologically. Electrocardiographic changes were detected at rest in 72% of the isoproterenol injected rats: pathological Q waves, lengthening of the QRS complex, and QRS abnormality were found in 50%, 44%, and 44% of these animals respectively. Ajmaline induced similar changes in both control and isoproterenol treated rats (P wave enlargement (p<0.01 and p<0.001 respectively), increased PR interval (p<0.003 and p<0.001 respectively), and increased QaT interval (p<0.001 in both groups)). However, ajmaline caused an increase in heart rate only in isoproterenol treated rats (p<0.05). A pronounced increase in PR interval was not observed in control rats. When the electrocardiographic and pathological findings were compared, the ECG changes were found to have 91% sensitivity, 83% specificity, and 91% positive predictive value. Thus it was concluded that the resting ECG is a reliable method of detecting myocardial lesions in isoproterenol-injected rats.

Plants that maintain the histo architecture and electrocardiographic tracings in experimental myocardial infarction were extensively reviewed and presented here.

Thippeswamy et al. designed a study to evaluate the cardioprotective potential of ethanol extract of Cucumis trigonus Roxb fruit (CTE) on the basis of
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electrocardiographic, biochemical and histopathological parameters in isoproterenol induced myocardial infarction in rats. Male albino sprague dawely rats were pretreated with CTE (75 and 150 mg kg⁻¹) daily for a period of 14 days. After the treatment period, ISO (200 mg kg⁻¹) was subcutaneously injected to rats at an interval of 24 h for two days to induce myocardial injury. Animals treated with ISO showed significant elevation in ST segment, reduction in P wave, QRS complex and R-R interval. In addition there was an increase in heart rate, prolongation of QT interval and cardiac cycles compared to normal control animals. Pretreatment of CTE (75 and 150 mg kg⁻¹) for 14 days and two doses of ISO (200 mg kg⁻¹) administered rats exhibited normal ECG pattern. The observed results were further confirmed by histopathological findings. Animals treated with CTE demonstrated marked improvement in ISO-induced alterations such as vacuolar changes, edema, capillary dilatation and leukocyte infiltration.

S-allylcysteine (SAC) is an organosulfur-containing compound derived from garlic. Studies have shown that garlic is beneficial in the treatment of cardiovascular diseases. Chuah et al. aimed to elucidate the cardioprotection of SAC using acute myocardial infarction (AMI) rat models. The propargylglycine (PAG)-treated MI group showed severe edema in the infarct zone, with inflammatory cells distributed throughout. The myocardium of the SAC-treated MI group showed mild edema with few inflammatory cells and the morphology of the myocardial tissue was better preserved compared with the PAG treated group. All groups showed significant ST elevation, which is characteristic of MI. The SAC-treated group had a less elevated ST-segment. In conclusion, this study provided novel evidence that SAC is protective in myocardial infarction via an H2S-related pathway.

Ithayarasi et al. studied the effect of alpha-tocopherol on isoproterenol-induced myocardial infarction in rats-with reference to electrocardiographic biochemical and histological evidences. The effect of alpha-tocopherol (6 mg/100 g body wt, orally, daily for 90 days) pretreatment in isoproterenol (20mg/100 g body wt, subcutaneously, twice at an interval of two days at the end of the alpha-tocopherol
pretreatment) induced myocardial infarction was studied in rats. Isoproterenol administered rats showed electrocardiographic changes suggestive of myocardial infarction with marked ST segment elevation, Q waves appearance and a significant increase in heart rate. The histology of heart and aorta showed marked fragmentation of muscle fibres and necrotic lesions in isoproterenol administered rats. Alpha-Tocopherol pretreated rats showed a near normal ECG pattern, and a near normal histology of heart and aorta.

Considerable ischemic alterations were observed in the animals treated with isoproterenol, including areas of myocardial necrosis, contraction band necrosis, increased plasma levels of cardiac necrosis markers and electrocardiographic modifications (ST segment changes and T wave inversion). The myocardial infarction was attributed to the inotropic activity of isoproterenol leading to intracellular calcium overload. The cardiac necrosis phenomena appear to be associated with isoproterenol-induced lipid peroxide generation (as shown by the decrease in plasma Vitamin E levels) and increased procoagulant activity (a shortened PTT). Pinelli et al. evaluated the protective effect of propranolol or labetol on isoproterenol induced myocardial infarction. Pretreatment with propranolol or labetalol counteracted the appearance of the myocardial histological alterations and the associated ECG and biochemical lesions. This protective activity was attributed to the β-blockade.