CHAPTER I

Introduction and Review of Literature
INTRODUCTION

Cardiovascular disease, a multifactorial disease is the principal cause of death in countries throughout the world and is posing an increasing threat to our population, infiltrating into younger age groups and poor strata of the society. Despite the progress of its prevention by the advent of newer pharmacological approaches and changes in lifestyle, the incidences of cardiovascular disease (CVD) continues to be fast increasing. Indians constitute around \( \frac{1}{6} \)th of the humanity and have a much higher rate of cardiovascular diseases than other ethnic groups in the world.

Atherosclerosis is responsible for almost all cases of cardiovascular diseases and a number of factors are associated with the increased risk for atherosclerotic lesions in coronary arteries and other arterial beds. The overwhelming importance of atherosclerosis has stimulated enormous efforts to discover its cause. It is considered to be a chronic inflammatory response of the arterial wall initiated by some form of injury to the endothelium. High blood pressure physically stresses the arterial lining, while the circulating substances such as low-density lipoprotein (LDL) cholesterol, homocysteine, free radicals and nicotine chemically damage it. White blood cells then attach to the damaged wall and take up residence. Then for reasons that are not entirely clear, they begin to accumulate cholesterol and other fats. Platelets also latch on; smooth muscle cells
proliferate, releasing substances that cause the formation of fibrous tissue. The overall effect is a thickening of the arterial wall called a fibrous plaque.

An important mechanism of membrane damage in coronary artery disease is injury induced by free radicals, particularly by reactive oxygen species (ROS). Oxygen is essential for life, but around 5% or more of inhaled oxygen is converted into ROS such as superoxide anion radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl ions (OH-) by univalent reduction of O$_2$. Thus cells under aerobic conditions are always threatened with the insult of ROS, which is however efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. When the balance between ROS production and antioxidant defence is lost, ‘oxidative stress’ results, which through a series of events deregulates the cellular functions leading to various pharmacological conditions including cardiovascular dysfunction.$^5$

Chronic or repetitive endothelial injury is the cornerstone in atherogenesis and endothelial injury induced in experimental animals by mechanical denudation, haemodynamic forces, immune complex deposition, irradiation and chemicals causes intimal thickening and in the presence of high-lipid diets, typical atheromas.$^6$ Endothelial injury and dysfunction leads to increased permeability to plasma constituents like monocytes and platelets. Monocytes migrating into the subintimal space, transform into macrophages, which in turn ingest oxidised LDL (excess LDL in the plasma, which is basic to the formation of fatty streaks) to form ‘foam cells’.$^7$
Evidences gathered from many sources have linked the development of atheroma to the elevation of blood cholesterol level and that of lipoproteins. LDL is long been associated with atherosclerosis. It has been well documented that the oxidised or acetylated form of LDL is more atherogenic than the native ones. Other lipoprotein fractions such as very low-density lipoproteins (VLDL) and chylomicrons are also associated with premature atherosclerosis.

The prevention and treatment of atherosclerosis should target reduction in plasma LDL cholesterol levels by anti-lipidaemic drugs, inhibition of oxidative modification of LDL cholesterol with oxygen free radicals using antioxidants and other free radical scavengers, augmentation of high density lipoprotein (HDL) cholesterol mediated reverse cholesterol transport system, control of hypertension and diabetes mellitus, prevention of smoking and other lifestyle managements. Clinical trials and pathophysiological evidence support the use of aggressive therapy in patients with arteriosclerotic vascular disease and in those with several risk factors for the disease.

Although several chemicals and drugs are generally used to lower blood lipids and atherogenesis, the drugs of plant origin receives considerable attention to re-establish traditional claims with scientific interest. The currently used hypolipidaemic drugs lag behind the desired properties such as efficacy and safety in long-term use, cost and simplicity of administration. Most of the risk factors of coronary artery diseases can be taken care by simple lifestyle measures and judicious use of medicinal plant drugs, which are abundantly found in the
Indian subcontinent and have been found to be effective in numerous clinical and experimental studies.

Opinions about the safety, efficacy and appropriateness of medicinal plants vary widely among medical and health professionals in countries where herbal medicines are used. The problem is further complicated by the fact that many ‘plant medicines’ available in world trade often are sold as herbal medicinal preparations when they include non-herbal substances. These non-herbal additives often include toxic metals, poisonous substances, or refined prescription drugs.

The increased use of plant medicines has the potential for improving public health and lowering health care costs. Herbs are mines of medical agents and the need for research was felt to find efficacious, cheap and safe hypolipidaemic agents, from among the natural products.

*Commiphora mukul*, has been rediscovered in the field of research for treating high cholesterol levels. Guggul (gum resin of *Commiphora mukul*) has also been shown to reduce the stickiness of platelets - another effect that lowers the risk of coronary artery disease. Garlic is attributed to have fibrinolytic, antithrombotic, platelet antiaggregatory, hypolipidaemic and hypoglycaemic activities. *Plumbago* has been reported to possess antitumour, anticancer and hypocholesterolaemic activity.

Fruits of *Semecarpus anacardium* (Anacardiaceae), popularly known as ‘Ardha Vaidya’ have been used in Ayurveda against several types of ailments such as insanity, fever, dysentery, loss of appetite, neurological disorders, cancer
and cardiac troubles.\textsuperscript{15-16} \textit{Hemidesmus indicus} (Asclepiadaceae) has been used as folk medicine and as ingredient in Ayurvedic and Unani preparations against diseases of blood, inflammation etc.\textsuperscript{17} \textit{Terminalia arjuna}, commonly known as ‘arjun’ is an ever green tree, belonging to Compretaceae family, the bark of which is used as medicine in heart disease since 600 B.C.\textsuperscript{18}

\textit{Tinospora cordifolia} was used widely in Ayurvedic system of medicine and was categorized as ‘Rasayana’, meaning rejuvenative.\textsuperscript{19} This plant has been mentioned in literature as a general tonic and for its anti-inflammatory, anti-arthritic, anti-allergic, anti-malarial and anti-diabetic properties.\textsuperscript{20-21} The plant \textit{Withania somnifera} exhibited antioxidant, diuretic, antistressor, hypocholesterolaemic and hypoglycaemic effect.\textsuperscript{22-23} \textit{Ocimum sanctum} has been reported to possess, hypotensive, anti-inflammatory, immunomodulatory, analgesic, cardiac depressant and anti-stress activity.\textsuperscript{24-26}

Validating these earlier findings and traditional experience, present study was undertaken to formulate a polyherbal product, containing the extracts of selected plants. \textit{Commiphora mukul}, \textit{Allium sativum}, \textit{Plumbago indica}, \textit{Semecarpus anacardium}, \textit{Hemidesmus indicus}, \textit{Terminalia arjuna}, \textit{Tinospora cordifolia}, \textit{Withania somnifera} and \textit{Ocimum sanctum} were selected for the formulation. This formulation was used to evaluate its efficacy against cardiovascular diseases, in diet-induced atherosclerosis of rabbits.
REVIEW OF LITERATURE

Atherosclerosis and its sequelae of coronary artery disease and acute coronary syndromes have been the major cause of morbidity and mortality. It is estimated that half of all deaths in the world are attributed to atherosclerosis and its complications. Though atherosclerosis begins in early childhood; the condition usually progresses more rapidly in middle age and affects men more adversely than women. The most distressing feature of this non-communicable epidemic is its spread into the comparatively younger population involving middle and/or lower socio-economic strata of the society.

Atherosclerosis is a specific disease that affects arteries, especially large and medium sized arteries. The early manifestation of the disease process is the fatty streak. It consists of lipid-laden accumulations of smooth muscle cells, commonly found at vessel branch site. These lesions progress to fibrous plaque, a cholesterol and connective tissue rich deposit of smooth muscle cells projecting into the arterial lumen. The most advanced lesion – the ‘complicated lesion’ - is a fibrous plaque to which calcification, ulceration and haemorrhage has been added.

The early event in the disease process that leads to the formation of atheroma is known as atherogenesis. It involves the interplay of many blood borne elements with the arterial wall. An injury to the endothelial wall is known to be the primary event in atherogenesis.
Endothelial injury and dysfunction leads to increased permeability to plasma constituents like monocytes and platelets. Monocytes migrate to the subintimal space, transform into macrophages, which in turn ingest oxidised LDL to form 'foam cells'. The platelets that migrate into the intima secrete platelet-derived growth factor (PDGF), which recruits smooth muscle cells from media to the intima. The smooth muscle cells proliferate and release collagen, thromboplastin and proteoglycans essential for the formation of the 'plaque'. Macrophages release cytokines like interleukin-1 (IL-1), tumour necrosis factor (TNF), that propagate injury. Peroxidative injury increases the atherogenic potential of LDL, VLDL and intermediate density lipoproteins (IDL). A steep oxygen gradient in the arterial wall generates free radicals, which enable the formation of oxidised LDL. The figure (1) explains various steps in the pathogenesis of atherosclerosis.

Some of the lipoproteins such as LDL-1, VLDL, and Lipoprotein (a) [Lp (a)], are more likely to be associated with accelerated atherosclerosis. The cells, which cumulate in the intima like monocytes and macrophages, undergo autocrine conversion to PDGF synthesis stimulated by LDL from the hyperlipidaemic serum. Interstitial free radical and excess of oxidised LDL injure and destroy the 'foam cells', leading to the formation of necrotic extracellular lipid core, which is considered a key step in lesion progression. The Lp (a) may promote platelet aggregation and thrombus formation thus contributing to atherosclerotic plaque growth.
Fig.1: Schematic representation of pathogenesis of atherosclerosis

**ANTIOXIDANT POOL**

- Free radicals

**THROMBOSIS**

- Nitric oxide inactivation
- Altered fatty acid pool
- Release of cytokines

**INFLAMMATION**

- Homocysteine

**INFECTION**

- Endotoxin

**ENDOTHELIAL INJURY**

- Monocytes/macrophages
- Lipid incorporation, Free radicals
- NO, SMC Proliferation

**ATHEROSCLEROSIS**

NO - Nitric oxide, SMC - Smooth muscle cell,
PMNS - Polymorphonuclear leukocytes
(Reproduced from35)
A series of non-enzymatic (e.g., vitamin E and A) and enzymatic substances (antioxidant enzymes) act as free radical scavenging systems and break down reactive oxygen species, such as superoxide anion radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl ions ($OH^-$), thereby reducing the free radical mediated injury (Figure 2). The antioxidant enzymes are localized in the cellular organelles and include Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx).

Elevated levels of cholesterol (lipids) can induce turbulent flow, producing membrane impermeability and sometimes injury to the endothelium. Evidences gathered from many sources have linked the development of atheroma to the elevation of blood cholesterol levels, primarily, LDL cholesterols, VLDL cholesterols and chylomicrons. Hence, knowing and controlling cholesterol is an important step in preventing the disease.

Furthermore a number of factors often acting in concert are associated with atherosclerotic lesions in coronary arteries and other arterial beds. The term risk factor describes those characteristics found in healthy individuals, which are independently related to the subsequent occurrence of coronary vascular diseases. It includes modifiable lifestyles and biochemical/physiological characteristics as well as non-modifiable personal characteristics, such as age, gender and family history of early-onset coronary artery diseases. The role of various factors responsible for atherosclerosis is briefly described below.
Free radicals generated from oxidative stress react with LDL particles, thus oxidising vitE and the lipid component of LDL. The antioxidant systems prevent LDL oxidation by reducing oxidising compounds. Apo B-100 - Apolipoprotein B-100, PUFA - Poly unsaturated fatty acids, Gssg - Oxidised glutathione, Gsh - Glutathione. (Reproduced from38)
Lifestyles and CVD

Diet and habits

Diet is an important determinant of CVD risk and is mediated through the influence of biological risks, such as lipids, blood pressure (BP) and obesity. Saturated fatty acids in the diet increase LDL cholesterol levels, while monounsaturated fatty acids lower LDL cholesterol and do not affect HDL cholesterol.41

Sedentary habit is a risk factor for atheromatous disease, and moderate physical activity may be having a beneficial effect on cardiovascular morbidity and mortality, partly mediated through its influence on the traditional cardiovascular risk factors, such as body weight, lipids and BP.42-43

Cigarette Smoking

There is strong evidence that smoking can significantly increase CVD mortality and morbidity; an adverse effect related to the amount of tobacco smoked daily and the duration of smoking.44-45 Passive smoking has also been found to increase CVD risk and the impact of smoking on CVD risk has modified by plasma lipid levels.46 Nicotine stimulates release of adrenaline leading to increased serum concentration of the free fatty acids.47 Free fatty acid is a stimulant of hepatic secretion of LDL and triglycerides (TG). The free fatty acid can also stimulate hepatic synthesis and release of cholesterol.48 In addition to this, cigarette smoking can alter coagulation system, produce various free radicals;
all of which may contribute to atherosclerosis. The benefits of smoking cessation are seen regardless of how long and how much the person previously smoked.44,46,49

**Alcohol consumption**

Epidemiological data indicate that moderate alcohol intake has a protective effect on CVD.50 Alcohol also appears to reduce the risk of peripheral arterial disease among apparently healthy men. Benefits from alcohol appear to be mediated mostly by an elevation in serum HDL cholesterol as well as by the effects of alcohol on platelets and fibrinolysis.51 However, increased alcohol consumption was associated with an increased risk of cardiovascular disease due to hypertension and haemorrhagic stroke or sudden arrhythmic death.52-53

**Biochemical / Physiological characteristics and CVD**

**Hypertension**

The importance of elevated BP as a risk factor for CVD, heart failure, cerebrovascular disease and renal failure in both men and women has been clarified in a large number of epidemiological studies.54-55 Systolic BP is at least as powerful a coronary risk factor as the diastolic BP, and isolated systolic hypertension is also established as a major hazard for coronary disease and stroke.56-57 Clinical trials of BP lowering using different drugs have clearly shown that the risks associated with increased BP can be substantially reduced, especially for stroke, but also for CVD and heart failure: a goal BP of <140/90 mm Hg is appropriate for primary and secondary prevention.58-59
Dyslipidaemia

Diets rich in cholesterol and saturated fats contribute to the elevation of lipid (fat) levels in blood and to the progression of atherosclerosis. Epidemiological observations have shown that there is a strong positive relationship between the concentration of circulating cholesterol, specifically the LDL cholesterol fraction and the risk of atheroma. This relationship is non-linear and depends strongly on the presence of other risk factors including male sex, arterial hypertension, cigarette smoking, diabetes mellitus and positive familial or personal history of ischaemic heart disease, electrocardiographic and echocardiographic observations.

Approximately two-thirds of cholesterol circulating in the blood is made up in the liver. Hepatocytes synthesize cholesterol and bile acids from acetate, and secrete them in bile into the intestine where they are involved in fat absorption. The rate-limiting enzyme in the cholesterol biosynthesis is the 3-hydroxyl 3-methyl glutaryl Coenzyme A (HMG CoA) reductase. Fats absorbed in the form of TG-rich chylomicrons and free fatty acids are cleaved from TG by lipoprotein lipase (LPL), an enzyme on the surface of the endothelial cells. Chylomicron remnants are taken up by hepatocytes to complete the exogenous cycle. The endogenous cycle consists of the secretion of TG rich lipoprotein particles (VLDL) that also contain cholesterol, by the liver into the blood, followed by the removal of free fatty acids by LDL in the capillaries. This results in progressive enrichment of the particles with cholesterol with an increase in their density through IDL to LDL. This circulating LDL cholesterol is especially
atherogenic. LDL cholesterol particles bind to receptors located in coated pits on the surface of hepatocytes, hence the plasma concentration of LDL cholesterol is determined by a balance between LDL synthesis and hepatic uptake.

LDL cholesterol consists of about 2,000 molecules of cholesterol, 1,000 molecules of phospholipid and one large protein on the surface called apolipoprotein B-100 (Apo B-100). High levels of LDL cholesterol are so firmly established as a risk for coronary heart disease that many people refer to it as 'bad cholesterol'. This epithet distinguishes it from the 'good cholesterol' in high-density lipoproteins (HDL), the blood levels of which are inversely related to the risk of coronary heart disease. Despite the stigma it bears, the LDL cholesterol plays a vital role in transporting cholesterol throughout the body. Cholesterol is an essential component of all cell membranes; also, the adrenal glands and gonads use it to manufacture steroid hormones such as testosterone. To obtain cholesterol, cells take up LDL cholesterol from the blood using LDL receptors, which bind to the Apo B-100 on the lipoprotein surface. A genetic deficiency in LDL receptors leads to elevated blood cholesterol and an enhanced risk for coronary heart disease. Diets high in cholesterol and saturated fats could stimulate this defect in genetically normal persons; such diets signal the body to produce fewer LDL receptors. A decrease in the number of LDL receptors prolongs the circulation of LDL particles in the blood stream - an outcome that increase the chance that the particles and the cholesterol they contain will be incorporated into atherosclerotic plaques.
HDL cholesterol does not cause such problems, because, unlike LDL cholesterol, it favours the delivery of excess cholesterol from peripheral sites to the liver for elimination. The mechanism for the protective effect of HDL cholesterol against atherosclerosis is unknown, but it may relate to the action of HDL cholesterol in the reverse cholesterol transport or to its ability to act as an antioxidant and has been shown to inhibit lipid peroxidation. HDL cholesterol contains platelet activating factors acetyl hydrolase and paraoxonase enzymes, which may protect against the formation of biologically active oxidised LDL particles. These contrasting mechanisms provide a rationale for the association of risks for coronary heart disease with high LDL levels and low HDL levels.

There is strong and positive association between total and LDL cholesterol and risk of cardiovascular events extending over a wide range of cholesterol concentration. The association applies to individuals with or without established CVD as well as to women and men. Other risk factors can substantially aggravate the effects of LDL cholesterol. The results of epidemiological studies, as well as trials with angiographic or clinical end points confirm the importance of LDL in the pathogenesis of atherosclerosis. There is also strong and inverse association between HDL cholesterol and the risk of CVD in both men and women and in subjects with or without established CVD; the lower the concentration of HDL cholesterol the greater the risk of CVD.

**Glucose intolerance and Diabetes**

Insulin resistance, hyperinsulinemia and glucose intolerance appear to promote atherosclerosis. Furthermore, both major types of diabetes mellitus
(Type I and II) are associated with a markedly increased risk of CVD, cerebrovascular and peripheral vascular diseases. Diabetes is a particularly strong cardiovascular risk factor in women, and decreases the relative protection of the female gender against atherosclerosis. The excess cardiovascular risk associated with diabetes is partly explained by the adverse effects of diabetes on other risk factors, such as hypertension, dyslipidaemia, hyper fibrinogenaemia etc., but may also be related to the direct effects of hyperglycaemia or the diabetic state itself.

**Obesity**

Prospective epidemiological studies in western populations have shown that body weight expressed in relation to height is related to cardiovascular mortality. Obesity has an adverse influence on a number of other vascular risk factors, including BP, lipids and glucose tolerance, which could partly explain effects on CVD risk. Furthermore, obesity was independently associated with left ventricular hypertrophy, while weight loss can reduce left ventricular mass. It should be mentioned that central obesity with an increased intraobesity fat mass is associated with a particularly adverse effect on these risk factors and is also linked to insulin resistance.

**Other Cardiovascular Risk Factors**

In addition to the established risk factors, several other variables have been identified as predictors of vascular disease
Left ventricular hypertrophy (LVH)

LVH is defined as a left ventricular mass exceeding 131g/m² of the body surface area in men and 100g/m² in women and is the response of the heart to chronic pressure or volume overload. Its incidence increases with age, BP, and obesity. LVH is independently associated with increased incidence of cardiovascular disease, and cause mortality and stroke. Effective blood pressure control in hypertensive patients, along with non-pharmacological interventions such as weight reduction, sodium restriction and aerobic physical exercise, can reduce left ventricular mass.

Hyperhomocysteinaemia

A number of epidemiological and observational studies have suggested that increased fasting total homocysteine levels (>15 m mol/l) are independently associated with CVD, myocardial infarction, peripheral vascular disease, cerebrovascular disease, stroke, cardiac allograft vasculopathy and CVD death. Genetic and nutritional factors, such as deficiencies in folate, vitamin B₁₂ and vitamin B₆ are associated with increased serum levels of homocysteine, while in patients with hyperhomocysteinaemia; supplementation with these vitamins can decrease homocysteine levels.

Lipoprotein (a) [Lp (a)]

Some prospective and retrospective studies have suggested an independent risk association between the increased Lp (a) levels (>30 mg/dl) and the presence of cardiovascular disease, myocardial infarction and cerebrovascular
Many experimental trials have confirmed a positive correlation between Lp (a) and atherosclerosis. It is assumed that ¼ of the heart attacks in men below 60 years of age occur in those who have inherited high concentration of Lp (a). In hyperlipidaemic patients with increased Lp (a) levels, the decrease in LDL cholesterol levels is followed by neutralisation of the atherogenic potential of Lp (a).

Lp (a) turns out to be quiet similar in structure to LDL. It contains cholesterol, phospholipids and one molecule of Apo B-100. Its distinguishing feature is the presence of one additional larger protein, dubbed apolipoprotein (a) [Apo (a)] that is chemically linked to Apo B-100. The Apo (a) molecule is homologous to plasminogen. It is possible that Lp (a) might bind and prevent fibrinolysis either by blocking activation of plasminogen or by preventing initial access and binding of plasminogen. Lp (a), particularly after oxidation can stick to macrophages and promote their transformation into foam cells. In addition it is thought to be mitogenic towards the cells in vessel walls similar to hepatocyte growth factor. Lp (a) is now recognized as the most powerful and most prevalent risk factor for premature atherosclerosis in diverse population. It is ten times as atherogenic as LDL. In addition it has significant thrombogenic and antifibrinolytic properties due to structural similarity to plasminogen.

**Hypertriglyceridaemia**

The exact role of increased TG levels as a risk factor for atherosclerosis is not clear. However, in multivariate analysis after adjustment for other risk factors, especially HDL cholesterol, the predictive power of the
increased TG is substantially reduced. Furthermore there is significant variation on fasting TG. Interestingly indirect data from prospective clinical trials have pointed out that the reduction of TG results in a significant decrease in CVD events.77

Major TG carrying lipoproteins in the plasma is chylomicrons and VLDL. Chylomicrons are the carriers of exogenous (dietary) TG and VLDL are the carriers of endogenous TG synthesised in the liver. The central core of chylomicrons contain major part triacylglycerol and trace amount of cholesterol. Primary causes of high TG include familial hypercholesterolaemia and secondary effects can arise due to carbohydrate, alcohol, and diabetes induced high TG, obesity, chronic renal failure, nephritic syndrome, excessive stress, etc.78

**Increased fibrinogen level and other thrombogenic factors**

Studies have clearly shown the association of increased plasma fibrinogen level with cardiovascular disease; reduction of increased fibrinogen levels in CVD patients could decrease the incidence of cardiac death and ischaemic heart disease. It should be mentioned that smoking cessation, weight loss, regular exercise, moderate alcohol consumption and fibrates could significantly reduce plasma fibrinogen levels.79

Several other factors participating in blood coagulation have been associated with CVD risk, including factor VII levels, plasminogen activator inhibition and increased platelet aggregation. Compelling evidence from randomised controlled trials now exists on the beneficial effect of antiplatelet
agents (mainly aspirin) in the prevention of cardiovascular events in patients with established cardiovascular disease.\textsuperscript{80}

**Oxidative Stress**

Oxidative stress and further modification of LDL plays an important role in the atherosclerotic process \textsuperscript{81} and is initiated by the oxidation of lipids in LDL, also termed lipid peroxidation. The minimally modified LDL (oxidised LDL) induces local vascular cells to produce monocyte, granulocyte and macrophage colony recruitment and differentiation to macrophages in arterial walls. The accumulating monocytes and macrophages stimulate further peroxidation of LDL.

Epidemiological and observational studies have shown that intake of antioxidant vitamins (A, C and E) may provide protection against cardiovascular disease. Specifically there is evidence that plaque stability, vasomotor function, and the tendency to thrombosis are subject to modification by specific antioxidants.\textsuperscript{82} It is worth mentioning that dietary modification, such as monounsaturated fatty acids (as well as flavonoids) present in red wine; vegetables, fruits and tea could significantly reduce the oxidative stress.\textsuperscript{83}

**Infectious agents**

Recent data suggest the potential links between infectious agents and cardiovascular diseases. Numerous infectious agents have been considered as possible causes of vascular injury and inflammation for the development of vascular diseases. Viral agents, especially those belonging to herpesviridae family, *Cytomegalovirus* (CMV), *Herpes simplex virus* (HSV) and *Epstein-Barr*
virus (EBV) have been investigated extensively. Bacterial agents, such as
Chlamydia pneumoniae (CP), Helicobacter pyroli (HP) have also been
implicated. However there are a number of questions concerning the extent to
which these microorganisms play a role in the pathogenesis of atherosclerosis.

Inflammation

It has recently been stated that atherosclerosis is clearly an
inflammatory disease and does not result simply from the accumulation of lipids.
Evidences linking inflammation to atherosclerosis stems from studies focusing
on acute and chronic phases of CVD. Thus markers of inflammation, such as
C-reactive protein, have recently been described as a potential predictor for future
cardiovascular and cerebrovascular events. Furthermore, elevated acute-phase
reactants and cytokine production with a focal predominance of inflammatory
cells have been found in patients with unstable coronary syndromes. The
circulating levels of these inflammatory markers are altered by treatment with
lipid lowering drugs, such as pravastatin.

Therapeutic strategies in CVD

Striking advances in the understanding of atherosclerosis and its
prevention have afforded many opportunities for the development of therapeutic
interventions for treatment. Even when an atherosclerotic lesion is well advanced
and the risk of catastrophic plaque rupture is high, lipid-lowering therapy may
stabilize the plaque and dramatically reduce the risk of thrombotic events. The
reduction in clinical events was attributed to stabilization of the fibrous cap of the
atherosclerotic lesion thus protecting it from rupture.\textsuperscript{87} Projections based on the results of recent large lipid lowering intervention trials, and from established benefits of cessation of smoking and treatment of hypertension, allow the prediction that a major impact on coronary heart disease is achievable. Central to the pathogenesis of atherosclerosis is the deposition of cholesterol in the arterial wall. Nearly all lipoproteins are involved in this process, including cholesterol carried by VLDL, remnant lipoprotein \textsuperscript{88} and LDL.\textsuperscript{89} Keys to the prevention and treatment of cardiovascular disease are the elimination or modification of risk factors, if possible, in conjunction with treatment of specific lipid disorders.

Reducing serum LDL cholesterol below the target levels does not necessarily result in a proportional reduction in the risk of cardiovascular disease, because the attenuation of the cholesterol-heart disease relation at lower serum cholesterol concentrations.\textsuperscript{90} Improvement in vasomotor function occurs when lipoprotein levels are brought into the normal range, an added benefit for lipid lowering therapy. Dietary treatment of hyperlipidaemia is a necessary foundation of drug treatment. Treatment with natural antioxidants, ascorbic acid and tocopherol, also mitigates the defect, suggesting the defect mediated by oxidized lipoproteins.\textsuperscript{91}

The progressive loss of lipid from the plaque that occurs with treatment probably also contributes to the physical stability of the plaque. Thus, lipid-lowering therapy exerts a diminuend effect on both the long-term generation of atherosclerotic plaques and the events underlying acute occlusive coronary
dependent on the extent of perturbation of lipoprotein levels toward ideal values, provide a rational basis for aggressive therapy of hyperlipidaemia. Results from animal experiments have indicated that macrophage foam cells may be a relatively reversible component in atherosclerotic lesions.92

In 1966 Satyavati, 93 carried out studies on hyperlipidaemic rabbits and demonstrated that the crude gum from Commiphora mukul significantly lowered serum cholesterol and protected the animals from serum cholesterol induced atherosclerosis. Further studies corroborated the hypocholesterolaemic/hypolipidaemic action of the gum guggul in experimental animals like pigs, chick, rats, rabbits and man.94 Alcoholic, terpenoid and steroid fractions of Commiphora mukul have been investigated by several workers. The main activity has been found to be located in a steroid fraction of guggulu.12 The guggulu steroids also inhibited platelet aggregation,13 enhanced clotting time 95 and were effective in fibrinolysis.96 These finding lead to pharmacological and toxicological studies that showed this herbal remedy to be effective in humans, with no adverse effects and one of the best herbs for treatment of obesity.97

The active constituents of guggul, resin, volatile oil and gum significantly lowered serum triglycerides and cholesterol (LDL and VLDL cholesterol). At the same time it raised the level of HDL cholesterol.98-99 Clinical studies in India have consistently confirmed guggul extracts improve lipid levels in humans.100-102

Garlic has an international reputation for lowering blood pressure and blood cholesterol levels, generally improving health of the cardiovascular
system. It has been reported that essential oils extracted from garlic prevent fat-induced hypercholesterolaemia, enhance fibrinolytic activity and inhibit in vivo and in vitro platelet aggregation. Garlic is also attributed to have antithrombotic, platelet antiaggregatory, hypolipidaemic, antimicrobial, diuretic and hypoglycaemic activities.

Garlic powder supplementation to the cholesterol rich diet seemed more advantageous than aspirin because it had beneficial effect on blood clotting and plasma cholesterol level. Garlic treatment ameliorated hyperlipidaemia and renal damage in chronic nephrosis. The curative role of garlic in arteriosclerosis was evaluated by Augusti et al.

Plumbagin is a known isolate from the Plumbago species belonging to the family Plumbaginaceae. Plumbagin administered to hyperlipidaemic rabbits reduced serum total cholesterol and LDL cholesterol. Further, plumbagin treatment prevented the accumulation of cholesterol and TG in liver and aorta and regressed atheromatous plaques of thoracic and abdominal aortae. Administration of ethanol extract of Plumbago zeylanica root alone and in combination with vitamin E, significantly reduced serum total cholesterol, LDL cholesterol and TG levels in experimentally induced hyperlipidaemic rabbits.

The milk extract of Semecarpus anacardium was found to inhibit lipid peroxidation in rats. Feeding the extract of Semecarpus anacardium inhibited progression of atherosclerotic lesion and promoted plaque regression and further helped in mobilization of lipid especially cholesterol from the liver.
*Hemidesmus indicus*, a twining shrub has been used as folk medicine and as ingredient in Ayurvedic and Unani preparations against diseases of blood, inflammation, etc.\(^{17,130}\) The plant *Hemidesmus indicus* has been reported against syphilis, chronic rheumatism, urinary diseases, diarrhoea, hepatic toxicity and skin infections in folk remedies.\(^{131-132}\) *Hemidesmus indicus* was used to treat Viper venom (haemotoxic) induced lethality\(^ {133}\) and against hypercholesterolaemia in hyperlipidaemic rats.\(^ {134}\)

Antioxidants have been shown to decrease the progression of experimental atherosclerosis. Vitamin E was advocated as an effective treatment for heart disease, the administration retards LDL oxidation, inhibits smooth muscle cell proliferation, inhibits platelet aggregation, inhibits expression and function of adhesion molecules, decreases synthesis of leukotrienes and potentiates the release of prostacyclin through up-regulation of systolic phospholipase A2 and cyclooxygenase.\(^ {7}\) It has been suggested that antioxidants present in the fruit and vegetables provides protection against heart disease. Indeed supporting evidence for this comes from the WHO Monica project in which the plasma concentrations of cholesterol and antioxidants expressed negative correlation. Additional evidence for beneficial effect of dietary antioxidants (Vitamins C and E and carotene) on cardiovascular disease in humans has been reviewed.\(^ {135}\) It has been reported that in vitamin C deficient guinea pigs, the ground substance of the sub-endothelial space was disturbed and lipid deposited in the sub-endothelial space. The latter could be reversed in animals by providing vitamin C in the diet.\(^ {136}\)
Diallyl disulphide, a constituent of garlic oil is a potent hypolipidaemic and antioxidant agent catalysing the reduction of oxidants and preventing free radical generation.137 Garlic is effective as a natural agent for treatment of hypertension.138 Garlic compounds protect vascular endothelial cells from hydrogen peroxide induced oxidant injury and oxidised LDL induced injury.139-140 It has suggested that aged garlic extract is an effective antioxidant in preventing or treating disorders related to endothelial cell injury associated with free radicals.141

Both juice and essential oil of Allium sativum were found to have significant protective action against fat induced fibrinogen and decrease in fibrinolytic activity and coagulation time. The raw and boiled forms of Allium sativum were reported to decrease total serum cholesterol and it’s free and ester fractions in healthy (control), hyperlipidaemic and ischaemic heart patients.142-143

Antiplatelet therapy constitutes the best available tool for evaluating the role of platelets in clinical manifestations of atherosclerosis. Further it has been shown that essential oil and compounds isolated from garlic are inhibitors of platelet aggregation.144-145 Patients with simultaneous atherosclerotic involvement in different vascular beds, i.e. those with more extensive atherosclerotic disease and a higher risk for occlusive vascular events, may accrue the greatest benefit from anti-platelet therapy.146

Terminalia arjuna, commonly known as ‘arjun’ was well known against a number of diseases related to heart.147-153 This plant was found to modify various common coronary risk factors like obesity, hypertension and
diabetes mellitus without any side effects.\textsuperscript{154} \textit{Terminalia arjuna} extract increased the contractile force of rat isolated heart.\textsuperscript{155-156} The drug lowered systolic blood pressure and body mass index and increased HDL cholesterol. The beneficial effect was also noticed in the case of stable angina CVD patients using treadmill and echocardiographic parameters where an improvement in tolerance of exercise, blood pressure response and left ventricular ejection fraction was observed.\textsuperscript{151} It also induces coronary vasodilatation and hypotension and inhibits platelet aggregation.\textsuperscript{18} It delayed myocardial ischaemia in pre-treated animals. The stem bark of \textit{Terminalia arjuna} exhibited hypolipidaemic effect in cholesterol fed rabbits.\textsuperscript{157} The bark of \textit{Terminalia arjuna}, which reportedly possessed many cardiovascular activities, may offer newer avenues for the management of much prevalent CVD.\textsuperscript{158} Administration of aqueous extract in hypercholesterolaemic rabbits produced marked fall in cholesterol levels associated with decreased aortic and tissue atherosclerosis.\textsuperscript{159-160}

\textit{Tinospora cordifolia} was widely used in Ayurvedic medicine as a tonic, vitalizer, and remedy for metabolic disorders.\textsuperscript{161} Methanolic extract of \textit{Tinospora cordifolia} has been shown to inhibit lipid peroxidation, superoxide and hydroxyl radicals \textit{in vitro}.\textsuperscript{162} \textit{Tinospora cordifolia} was found to possess normalising activity against stress induced changes in hormonal activity and a powerful anti-inflammatory agent.\textsuperscript{163-164} \textit{Tinospora cordifolia} was also reported to possess antitoxic \textsuperscript{165} and immunomodulatory properties.\textsuperscript{166} Administration of the root extract of \textit{Tinospora cordifolia} has been reported to possess hypolipidaemic effect.\textsuperscript{167}
Withania somnifera was known for its varied therapeutic uses in Ayurvedic and Unani practices of India.\textsuperscript{15,168-169} The anti-inflammatory activity of Withania somnifera was noticed on chronic inflammatory reactions against carrageenan induced paw oedema in rats.\textsuperscript{170-171} The immunomodulatory activity of ashwagandha was reported with a significant rise in haemolytic antibody responses towards human erythrocytes.\textsuperscript{172-176} The plant also exhibited antioxidant, hypoglycaemic, diuretic, antistressor and hypocholesterolaemic effect.\textsuperscript{22-23,177-179} Ashwagandha was also reported to possess adaptogenic, cardioprotective and anticoagulant properties.\textsuperscript{180}

Ocimum sanctum fixed oil obtained from seeds; exhibited significant anti-inflammatory, antipyretic, analgesic antiarthritic and antiulcer activities without any noticeable toxicity.\textsuperscript{25,181-182} Administration of fresh leaves of Ocimum sanctum resulted in significant lowering serum total cholesterol, TG, phospholipids and LDL cholesterol levels and significant increase in the HDL cholesterol and total faecal sterol contents.\textsuperscript{183-184}

The Ocimum sanctum fixed oil can inhibit enhancement of the vascular/capillary permeability and leukocyte migration following inflammatory stimulus.\textsuperscript{185} Ocimum sanctum extract showed promising wound healing action and was found effective against growth of HIV virus.\textsuperscript{187} Pre-treatment with Ocimum sanctum brought back the stress altered changes to normal levels indicating its stress alleviating effect.\textsuperscript{24,188-189} Stress is known to produce immunosuppression and studies revealed that Ocimum sanctum had
immunomodulatory activity.\textsuperscript{26,190} The chemopreventive efficacy of \textit{Ocimum sanctum} seed oil may be partly attributable to its antioxidant properties.\textsuperscript{191-192}

Fish oil concentrates were given to patients with angiographically proven coronary artery disease. Omega-3 fatty acids moderately mitigated the course of coronary atherosclerosis by its significant effect on platelet behaviour.

**Drugs**

Individuals with coronary artery diseases should be encountered to reduce all the risk factors that might potentiate the development of atherosclerosis. Since hyperlipidaemia being the primary risk factor in atherosclerosis, drugs that reduce lipid levels finds wide acceptance as hypolipidaemic and antiatherogenic agents. The powerful agents available and the demonstrated synergy among a number of them allow achievement of optimal levels of individual lipoprotein species in most compliant patients who are tolerant of the medications. Ancillary measures such as diet, exercise, avoidance of cigarette smoking, and treatment of hypertension and diabetes also are important elements of the management.

**Drugs lowering plasma lipoproteins - Statins**

An encouraging development in the treatment of hypercholesterolaemia has been the introduction of a new class of fungal derived compounds that are potent competitive inhibitors of HMG CoA reductase, the rate limiting enzyme in the biosynthetic pathway of cholesterol. Drugs of the statin class are structurally similar to HMG-CoA, a precursor of cholesterol, are extremely effective in lowering plasma concentrations of LDL.\textsuperscript{193} These drugs
lower serum LDL cholesterol concentration by upregulating LDL-receptor activity as well as reducing the entry of LDL into the circulation.\textsuperscript{194} Given alone for primary or secondary prevention of heart disease, these drugs can reduce the incidence of coronary artery disease by 25-60%; reduce the risk of death from any cause by about 30%.

Therapy with a statin also reduces the risk of angina pectoris and cerebrovascular accidents and decreases the need for coronary artery bypass grafting and angioplasty.\textsuperscript{195} Statins lower serum TG concentration, fibrinogen level and viscosity.

The available statins useful in treating most of the major types of hyperlipidaemia include atrovastatin, fluvastatin, lovastatin, mevastatin, pravastatin and simvastatin. The kidneys eliminate statins and the most common adverse effects of statins are gastro-intestinal upset, fatigue, muscle aches and hepatitis. Cataracts have occurred in animals treated with high doses of lovastatin, simvastatin and fluvastatin but not in humans given any kind of statins.\textsuperscript{196} HMG CoA reductase inhibitors are completely ineffective in patients with homozygous familial hypercholesterolaemia (unable to make LDL receptors).

**Bile acid binding resins**

The first of these agents, cholestyramine was originally used to control pruritus in patients with elevated concentrations of plasma bile acid due to cholestasis. Bile-acid-binding resins are now largely used, as adjuncts to statin therapy for patients, in whom further lowering of serum cholesterol
concentrations are indicated. The available resins are cholestyramine and colestipol that are similar in their safety and efficacy.

Bile-acid-binding resins cause abdominal fullness, gas and constipation in 30 percent of the patients. It causes acidosis in children or in patients with renal failure because chloride ions are released in exchange for bile acid. Both resins may reduce the absorption of vitamin D and other fat-soluble vitamins, but this defect is negligible except possibly in children.

**Gemfibrozil**

This fibric acid derivative acts chiefly by the removal of TG from plasma but also decreases production of VLDL by the liver. Side effects include gastrointestinal symptoms, hepatic dysfunction, and myositis. While improving the removal of VLDL particles, gemfibrozil might increase LDL production in some patients. It is useful primarily in treating severe lipemia.

**Nicotinic acid derivatives**

Nicotinic acid in pharmacological doses lowers plasma TG by 30-50% and cholesterol by 10-20% and increases HDL. There is evidence from coronary drug project that it reduces the rate of re-infarction as well as the surrogate endpoints of plasma lipid concentrations. Reduction in lipid concentration occurs in all types of hyperlipoproteinemia.

Adverse effects of nicotinic acid and its derivatives include flushing, postural hypotension, pruritus, headache, nausea, vomiting, diarrhoea, epigastric pain and rashes. Other adverse effects include hepatic dysfunction, exacerbation of peptic ulcer, hyperuricemia, gout and an increase in blood glucose.
Probucol

Probucol is a synthetic lipophylic antioxidant related structurally to butylated hydroxytoluene. In man it causes a slight reduction in LDL cholesterol and has been used therapeutically for the past decade. Enthusiasm for the drug has been limited because probucol reduces HDL cholesterol even more than LDL cholesterol. However interest in probucol has been rekindled recently because of the possibility that it may retard atherosclerosis by antioxidant mechanisms that extend beyond its effects on plasma cholesterol concentration.

Probucol is well tolerated and there is little short-term toxicity. Mild and transient diarrhoea occurs in a minority of patients on starting treatment. The only potentially life-threatening effect is prolongation of the QT interval, which occurs in about 50% of the patients. For this reason probucol should be avoided in patients with prolonged QT interval or ventricular arrhythmias. Abdominal pain, flatulence and nausea are the other side effects.

Omega-3 fatty acids

Omega-marine triglycerides are effective in reducing plasma TG concentrations, but have no effect on cholesterol concentrations. They are used in severe hypertriglyceridaemia and may prevent pancreatitis. Side effects include occasional nausea and belching with a fishy after taste. Fish oil is contraindicated in patients with familial hypercholesterolaemia in whom it increases total circulating LDL cholesterol.
Animal model

Since atherosclerosis is a silent and asymptomatic disease until complications arise with thrombosis producing clinical symptoms, it is necessary to have animal models that reproduce the human disease in its early stages. Experimental models of vascular disease have enhanced the understanding of the pathophysiological processes leading to vascular obstruction in both spontaneous and accelerated atherosclerosis and thrombosis. Animal models have provided insights into the role of platelets, antioxidants, lipids, rennin-angiotensin system, cytokines and growth factors in the evolution and progression of atherosclerosis and thrombosis; and have suggested potential therapeutic interventions.

Experimental atherosclerosis was successfully induced in rabbits. Feeding pure cholesterol to rabbits could produce hypercholesterolaemia, hyperlipidaemia and atherosclerosis.\textsuperscript{199-200} Experimental models of vascular diseases have enhanced our understanding of the pathophysiological processes leading to vascular obstruction in both spontaneous and accelerated atherosclerosis and thrombosis.\textsuperscript{201} Significant advances in our understanding of vascular biology and pathology, as well as the interactions of blood borne cells, lipids and proteins with the vascular wall, have allowed us to formulate new experimental hypotheses and to design therapeutic strategies.

Clinical trials and pathophysiological evidence support the use of aggressive therapy in patients with arteriosclerotic vascular disease and in those with several risk factors for the disease. Combination therapy with lipid lowering drugs is advisable, especially in patients with combined hyperlipidaemia. Some
people with high cholesterol are able to reduce to safe levels by using combinations of dietary supplements that have been shown to lower serum cholesterol, protect against LDL cholesterol oxidation and reduce the risk of an abnormal arterial blood clot formation.

The effectiveness of any cholesterol reduction therapy varies considerably between individuals. However it is extremely helpful to examine the effect of any medication for the modification on risk factors and on overall cardiovascular risk. It should be mentioned that a number of drugs might be needed to deal with several risk factors that coexist in the same individual. Thus special attention should be given to the drugs, side effects, and most importantly to the drug-drug interactions in order to minimise the unfavourable consequences.

A combination of *Allium sativum*, *Allium cepa* and *Commiphora mukul* significantly prevented rise in serum cholesterol and serum TG caused by atherogenic diet and confer significant protection against diet-induced atherogenesis.202 ‘IMMU-21’, an Ayurvedic antistress adaptogen and immune modulator, is a polyherbal formulation of Indian herbs containing *Ocimum sanctum* and *Withania somnifera* as major constituents.203 ‘ZEE STRESS’ containing the extracts of *Withania somnifera* and *Ocimum sanctum* as major constituents, was used as antioxidant, immunomodulator, anti-stress agent and adaptogen, and protected the animals from stress-induced alterations in ECG and blood pressure profile.204

A combination of herbal drugs, *Commiphora mukul*, *Terminalia arjuna* and *Inula racemosa* (‘CTI’) was found to decrease serum cholesterol and
serum TG. The drug also lowered systolic as well as diastolic BP. Sandika, an indigenous drug formulation containing Commiphora mukul, Biswellia serrata, Strychnos nuxvomica and Semecarpus anacardium, was used for inflammation; possessing anti-inflammatory and antioxidant properties. Cardipro', a poly herbal formulation containing standard extracts of Terminalia arjuna, Emblica officinalis, Withania somnifera, Ocimum sanctum and Boerhavia diffusa has been clinically tried in the amelioration of angina symptoms. Abana', a herbomineral drug containing Terminalia arjuna as the main constituent, reduced blood pressure and body weight in rats. OB-200G', another poly herbal formulation containing aqueous extracts of Garcinia cambogia, Zingiber officinale, Piper longum, Gymnema sylvestre and the resin of Commiphora mukul was reported to posses thermogenic, hypocholesterolaemic, body weight lowering, antidiabetic and digestive stimulant properties. Hemidesmus indicus, Plumbago indica and Tinospora cordifolia were used for hypocholesterolaemic, anti-inflammatory, antistress, rejuvenative and immunostimulant properties.

The following conclusions can be made from the above review:

- Hyperlipidaemia is a major risk factor in atherosclerosis, and lipid lowering by diet and drug therapy will be beneficial.
- Cigarette smoking, hyperhomocysteinaemia and diabetes mellitus are risk factors of atherosclerotic cardiovascular disease.
- Lp (a) is an individual risk factor and its role in atherosclerosis is being found crucial. Hence reduction of Lp (a) level will reduce the risk.
• Free radicals play a major role in the initiation as well as progression of atherosclerosis. Free radical mediated endothelial injury as well as lipoprotein oxidation are crucial. This points out that antioxidant drugs will have great importance in the prevention of atherosclerosis.

• Coagulation system is involved in atherogenesis as well as in the final thrombotic event. Platelets play an important role in the thrombotic process. Thus it can be assumed that anticoagulant and antithrombotic drugs would have beneficial effect in atherosclerosis.

• There are a number of lipid lowering drugs in use and they exhibit wide side effects.

• New drugs thus need to be evaluated for reducing the risk of cardiovascular and cerebrovascular death.

It has been suggested that the best approach for the treatment of coronary artery diseases is to use combinations of medicinal plants, where most of the risk factors can be taken care by judicious use of plant drugs, abundantly found in the Indian subcontinent. Evidences for benefit with such combination comes from observational studies.211

In the present study, all the selected plants possessed hypolipidaemic and hypocholesterolaemic properties. Commiphora mukul, Allium sativum, Semecarpus anacardium, Hemidesmus indicus, Tinospora cordifolia, Terminalia arjuna, Withania somnifera and Ocimum sanctum were additionally, antiinflammatory and antioxidant agents.22, 24, 140, 178, 191, 210 and hypotensive agents.22, 24, 139, 164, 173, 178, 189
Commiphora mukul and Allium sativum were also known for fibrinolytic and platelet aggregation inhibitory properties. Ocimum sanctum, Withania somnifera and Tinospora cordifolia were also noticed as immunoboosters. Commiphora mukul was used as a slimming agent against obesity and for elevating the level of good (HDL) cholesterol. Terminalia arjuna was selected with additional antithrombotic, antianginal and cardioprotective effects. Hemidesmus indicus was selected as a blood purifier, Plumbago indica, for the anti-inflammatory action and Semecarpus anacardium, as the major plaque regressing agent.

Phytomedicines, if combined with the preventive model of medical practice, could be among the most cost effective, practical ways to shift the focus of modern cardiovascular disease treatment to prevention and cardio protection. The formulation selected has not only been shown to lower cholesterol, but also protect against cardiovascular diseases by mechanisms like inhibition of cholesterol oxidising free radicals and abnormal blood clots in the body.

From the above review it is clear that the herbal products are effective in ameliorating complications arising from atherosclerosis. Hence the present study was undertaken and the study included:

- A formulation from selected plants was made and screened for properties in relation to cardiovascular diseases.
- The in vitro antioxidant activity was assessed by the capacity of the formulation in scavenging superoxide anions, hydroxyl radicals and the inhibition of lipid peroxidation.
- Screening of anti-inflammatory efficacy of the formulation by carrageenan and formalin induced pedal oedema.
- Screening of antithrombotic efficacy by platelet aggregation inhibition and anticoagulant properties.
- The lipid clearing capacity was assessed by the role of the formulation in enhancing the release and activity of lipoprotein lipase enzyme.
- The animals were made hypercholesterolaemic by feeding on HFD and the hypolipidaemic/hypocholesterolaemic efficacy of the formulation was assessed by the estimation of serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and phospholipids in rats.
- The formulation was screened for toxicity effects on the vital organs heart, liver and kidney by assessing the biochemical parameters (ALP, GOT, GPT, LDH, GGT, urea and creatinine).
- The antiatherosclerotic efficacy of the formulation was assessed in rabbits.
- Study of in vivo antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase and catalase) and the rate of lipid peroxidation were also assessed.
- Study on enzymatic activities related to lipid metabolism (lipoprotein lipase releasing and HMG CoA reductase activity).
- Estimation of total cholesterol, phospholipids and triglycerides in tissues (heart, liver, kidney and aorta).
- Comparison of the therapeutic potential of the formulation with an herbal formulation available in the market, Liposem.