CHAPTER 1

REVIEW ON CHOLESTEROL AND ITS RELATED DISEASES

1.1 INTRODUCTION

Various concepts in the field of physics have long been playing a prominent role in devising a number of diagnosis and treatment strategies concerned with human sufferings. The contribution by Physics to Medical Sciences ranges from the earliest optical microscope to the latest Positron Emission Tomography (PET) for potential applications for early detection and treatment. At this juncture amalgamation of crystal growth, a branch of solid state physics, with a biological aspect namely stone formation (lithiasis) opens many avenues for a number of discoveries. In depth studies on the crystallization behaviour of biomolecules will certainly fetch more informations on the in vivo crystallization process and the ways by which such crystal formation can be inhibited or modified in the case of pathological conditions.

1.2 CRYSTAL DEPOSITION DISEASES

Deposition of crystals in biological ducts leads to pathological conditions such as stone formation in kidney and gallbladder and occlusion of the coronary artery leading to atherosclerosis. The presence of such microcrystals leads to marked tissue damage. Large crystal deposits can give rise to pathological conditions through simple mechanical effects such as blocking of the ducts and hardening or weakening flexible tissues.
It is necessary to understand the mechanism of nucleation and growth of large stones in such ducts and the cause of eventual obstruction. For this an exhaustive research is required to have a clear and comprehensive understanding of the biological crystallization process using both synthetic and animal model systems. This will be useful in adopting better treatment strategies against various lithiasis. Stones are in general, aggregates of many single crystals. They often consist of several different chemical species. The formation of such complex stones may be either due to the epitaxial nucleation and growth on crystals already present or due to the formation of new nuclei and growth in the surrounding fluid and finally become entangled with each other. Irrespective of the mode of growth, the interaction between the tissue matrix and the growing crystalline aggregates are important because it determines the morphology, size and distribution of the deposit by means of local variations of material concentration, precipitant concentration, temperature, nucleating surfaces and growth promoters or inhibitors.

Almost all the species which form into crystals in the body are either ionic in nature (sodium urate) or strongly hydrogen-bonded (cholesterol). In either case, the binding forces within the crystal are strong, and as a result, the deposits are considerably harder than the soft and elastic tissues surrounding them. Hence a loss of elasticity and compliance will occur in structures such as arterial walls or articular cartilage when they become calcified.

The recent acceleration of effort in the field of biological crystallization has been driven by a very strong need to understand their crystal structures and also to identify the factors responsible for the nucleation and growth. This will eventually help in devicing new methods to modify or inhibit the crystallization of stones found in the human body. Despite the fact that a number of studies concerning the nucleation and
growth of cholesterol have been carried out in model bile, there is still considerable uncertainty regarding the inhibition of cholesterol crystallization in vivo due to possible side effects of the drugs/compounds used. In this context the medicinal plants forge ahead because of the fact that they are not only curing the concerned disease but also are not giving rise to any side effect. There have been intense studies to investigate the effect of medicinal plants and ayurvedic herbal drug preparations in patients having high cholesterol but none on the crystallization (Awasthi et al 1994-95; Cour et al 1995; Grunwald et al 1993; Jahromi et al 1993; Krishnapillay et al 1993; Lansky et al 1993; Seetharamaiah and Chandrasekhara 1993; Singh et al 1993; Sugiyama et al 1994).

The main objective of the present investigation is to crystallize cholesterol and study the effect of medicinal plants on the crystallization of cholesterol in vitro. Cholesterol and its esters are important steroids which are responsible for atherosclerosis and gallstones in the human body. Bile acids are derivatives of cholesterol which are used as therapeutic agents for the dissolution of cholesterol gallstones. Cholic acid, one of the bile acids that exhibits polymorphism and intercalation phenomena is crystallized for the first time in the gel medium.

1.3 CHOLESTEROL

One of the least desirable calcification in the human body is the formation of mineral deposition in atherosclerotic plaques. These plaques principally consist of lipids such as cholesterol, cholesteryl esters, phospholipids and triglycerides (Small and Shipley 1974). The substance which has a nucleus containing four-ringed carbon skeleton of cyclopentanoperhydrophenanthrene is known as steroid. Cholesterol is the most prominent member of the steroid family and is an important component of many eukaryotic membranes. Cholesterol derives its name
from a Greek word "Cholesterine" which means bile solid as it was first isolated from human gallstones. The systematic name for cholesterol is cholest-5-en-3β-ol. The molecular formula for cholesterol is $C_{27}H_{46}O$.

Cholesterol is an essential constituent of all animal cells. The lipid bilayer forms the cell membrane of almost all eukaryotic cells and is composed mainly of phospholipids. The fluidity of the cell contents is maintained by the presence of cholesterol within the bilayer. Any alteration in the membrane cholesterol would result in the altered cell function. This fatty substance is a life giving, life sustaining force. As potent as it is to maintain life when it is in proper balance - it is equally threatening to life when it is out of balance. Human beings accumulate cholesterol in two ways namely: by biosynthesis *in vivo* and through exogenous sources i.e., dietary cholesterol obtained from various animal fats and a minor quantity from vegetable sources.

In addition, cholesterol is the precursor of the other two major classes of steroids namely the steroid hormones and the bile acids. The male sex hormones such as testosterone and androgen and the female sex hormones such as progesterone and oestrogen are the important sex hormones which are the derivatives of cholesterol. Steroid hormones play a key role in the regulation of metabolism. These hormones come in a rich variety, each interacting in a highly specific manner with a receptor protein to effect gene expression in the appropriate target tissue. Bile acids are the primary degradation product of cholesterol. The bile acids are synthesized in the liver, stored in the gall bladder and secreted into the small intestine. Bile acids aid in the solubilization of lipids and thus facilitating their digestion by intestinal lipases. The important bile acids are: taurocholic acid, lithocholic acid and chenodeoxycholic acid.
Practically cholesterol is insoluble in water and is readily soluble in solutions containing amphiphilic compounds. It is a fatty substance, large concentration of it are found in the liver, brain and spinal cord.

1.3.1 Discovery of cholesterol

The study of gallstones during the latter half of the eighteenth century led to the discovery that the major constituent of most human gallstones is a white crystalline substance soluble in alcohol and ether. In the year 1816 Chevreul named this substance as cholesterine but the name cholesterol was adopted by French and English workers when Berthelot showed that cholesterine was an alcohol in the year 1859. By 1840s the compound now known as cholesterol had been shown to be a normal constituent of many animal tissues and in 1843 Vogel showed that it was present in the atheromatous lesions of human arteries.

1.3.2 Structure of cholesterol

Cholesterol is composed of the characteristic cyclopentanoperhydrophenanthrene ring. The structure of cholesterol is shown in Fig. 1.1 and has the following features:

i) Cyclopentanoperhydrophenanthrene ring system

ii) Two methyl groups, one attached to C\textsubscript{18} and the other attached to C\textsubscript{16}

iii) An aliphatic side chain attached to C\textsubscript{17}

iv) A OH group attached to C\textsubscript{3}

v) A double bond between C\textsubscript{5} and C\textsubscript{6}

Cholesterol is an unsaturated alcohol because of the presence of double bond between C\textsubscript{5} and C\textsubscript{6}. Cholesterol monohydrate (C\textsubscript{27}H\textsubscript{46}O\textsubscript{2}H\textsubscript{2}O)
Figure 1.1 Structure of cholesterol.
belongs to triclinic system having space group P1, with \(a=12.39(3), b=12.41(3), c=34.36(6)\text{Å}; \alpha=91.9(1), \beta=98.1(1), \gamma=100.8(1)°\) (Craven 1979).

### 1.3.3 Biosynthesis of cholesterol

Man and animals are capable of synthesizing cholesterol in various organ tissues. Liver is the most important site of cholesterol biosynthesis, although other sites include the adrenal glands, reproductive organs, skin and intestines. By means of isotopic techniques, it has been proved that acetate can serve as the starting material and by reacting with coenzyme A(CoA) (Myant 1981) it gives rise to cholesterol in the following sequence:

* Biosynthesis of cholesterol begins with acetyl-coenzyme A (acetyl CoA). Two molecules of acetyl CoA condense to form one molecule of acetoacetyl CoA.

* Acetoacetyl CoA links with one more molecule of acetyl-CoA to form β-hydroxy β-methyl glutaryl-CoA (HMG-CoA). This reaction is catalysed by a synthetase.

* HMG CoA is reduced by the enzyme HMG-CoA reductase and two molecules of nicotinamide adenine dinucleotide phosphate (NADPH) in two stages to form mevalonic acid.

* Mevalonic acid is phosphorylated three times in succession by using ATP, forming first a monophosphomevalonic acid, next a diphosphomevalonic acid and finally triphosphorylated mevalonic acid, a transient intermediate which simultaneously loses a molecule of phosphate and CO₂ to form isopentyl pyrophosphate, also known as the isoprene unit.
* The isoprene unit after isomerisation and condensation forms farnesyl pyrophosphate.

* Farnesyl pyrophosphate forms squalene.

* Squalene is cyclised to lanosterol.

* Removal of three methyl groups from lanosterol forms zymosterol, a compound which differs from cholesterol in the position of the double bond in the ring system and in the position of a double bond in the side chain.

* Movement of the double bond in the ring system of zymosterol to the normal position as in cholesterol would produce desmosterol, which differs from cholesterol only in the possession of an unsaturated linkage in the side chain. Desmosterol is the immediate precursor of cholesterol.

* Reduction of double bond in the side chain of desmosterol gives rise to the final product cholesterol.

The speed of cholesterol biosynthesis is chiefly regulated by the liver, largely depending on the exogenous supply in the whole body, the more cholesterol in the diet (exogenous), the less will be the biosynthesis (endogenous).

1.3.4 **Physical properties of cholesterol**

* Cholesterol forms white shining rhombic platelike crystals, when they are seen under microscope.
* The melting point of cholesterol is 150.7 ± 0.8°C.

* Cholesterol is sparingly soluble in water and soluble in organic solvents.

* When mixed with fat or oil, cholesterol has a peculiar property of enabling the fat or oil to absorb water. Lanolin is a greasy substance containing cholesterol, which enables lanolin to absorb water readily. Lanolin is used in pharmacy as the greasy vehicle in the preparation of ointments containing water soluble constituents.

* Cholesterol is a poor conductor of electricity, and serves as the insulator for electric discharge. It is probable that cholesterol found abundantly in brain acts as an insulator against nerve impulses which are electrical in character.

* Cholesterol exhibits liquid crystalline behaviour.

1.4 CHOLESTEROL LEVELS IN HUMAN BODY

The normal level of cholesterol in blood plasma is 160-250 mg per 100 ml. However the maximum level can be upto 320 mg/100 ml for people from western countries.

The plasma cholesterol in adult women is influenced by the menstrual cycle, pregnancy and the menopause. It has been shown that age and sex affects serum lipid levels in man. Diet and particularly dietary fat are probably related to serum lipid concentrations.
The anomalies in the cholesterol contents of blood and organs will be discussed in the following order:

1. Hypercholesteremia
2. Hypocholesteremia
3. Cholesterol deposition

The inter-relation of cholesterol and its solubility with other colloidal serum constituents proves that it is desirable to consider cholesterol saturation level rather than actual cholesterol content in the blood. The tendency of cholesterol to form pathological deposits is actually dependent on the cholesterol saturation index and also on the level of inhibitors and promoters present in the bile.

1.4.1 Hypercholesteremia

Hypercholesteremia may be considered a common finding in most patients with early coronary atherosclerosis. It is also observed in diabetes mellitus and xanthomatosis. The level of cholesterol rises due to the following factors:

i) nonavailability of sufficient quantity of bile
ii) renal complications
iii) hypothyroidism
iv) nephrosis and
v) hyperlipidemia.
1.4.2 Hypocholesteremia

The low level of cholesterol is associated with the prolonged starvation. It is also observed among the patients suffering from hyperthyroidism, Addison's disease, pancreatic and liver diseases.

1.5 DISEASES DUE TO CHOLESTEROL DEPOSITION

Cholesterol is deposited in a variety of tissues under diverse pathological conditions that may or may not disturb the normal functioning of the organisms. Cholesterol is deposited in atheromatous lesions and in xanthomatous lesions. The crystalline form of cholesterol is observed in gallstones (Admirand and Small 1968; Katz et al 1976).

1.6 GALLSTONES

The formation of gallstones technically known as cholelithiasis is a disturbance to the biliary tract. The stones are found in the gall bladder or in one of the bile ducts. The main components of gallstones are cholesterol and bilirubin, with small amounts of calcium carbonate and trace amounts of a number of elements such as sodium, magnesium, copper, aluminium, silicon, manganese, bismuth, iron and lead (Rajagopal et al 1988). Depending upon the major components present in the gallstones, they are divided into two types namely cholesterol and pigment gallstones (Sadaruddin and Zuberi 1987). High risk candidates for gallstones are overweight women after pregnancy, middle aged men and women with a high blood cholesterol level and the diabetics.
1.6.1 Gallstones prevalence

Important geographical and racial variations in the prevalence of gallstones have been studied by several workers (Bateson 1986). International variation becomes much more marked when African rates are compared to those in western nations. Gallstones are so uncommon in Africa that in some areas they are virtually never seen and the most recorded rates are less than 1.0%. The degree of urbanization appears to be very important in disease patterns in Africa. Detailed studies have not been carried out in Asia. From the available data, prevalences are found among the Singapore opium addicts. Japanese studies present an interesting epidemiological feature. In western civilization the gallstones are composed mainly of crystalline cholesterol while the stones in Asia are almost pigment. Japan occupies an intermediate position and both types of stones are found to occur. Nakayama and Miyaka (1970) confirmed that the composition of gallstones in Japan is gradually changing from the once predominant pigment stone to cholesterol stones. The prevalence of gallstone disease has increased over a similar period from 1.7% to 6.7%. The increasing urbanization and changing food habits that had taken place in Japan since the second world war were blamed for the changes in character of gallstone disease.

The American-Indian is particularly prone to developing cholesterol stones. As more epidemiological research is being carried out in South America, it is becoming evident that cholesterol gallstones are very common. A Chilean study in 1960-1971 showed that 35.2% of people over the age of 20 years in Sandiego had gallstones (Marinovic et al 1972). It is also interesting to note that the variation in the prevalence of gallstones has been observed in different geographical locations within a country.
1.6.2 Nucleation, growth and pathogenesis of cholesterol crystal

The gallstone formation is governed by various factors as seen in Fig. 1.2 (Bateson 1986). The most important factor is the saturation index of cholesterol with respect to bile.

There are three different stages by which cholesterol contributes to the pathogenesis of gallstones.

1. Cholesterol supersaturation
2. Cholesterol crystal nucleation and
3. Cholesterol crystal growth.

1.6.3 Cholesterol supersaturation

In general, 'supersaturation' of cholesterol in bile is a necessary condition for the nucleation and crystallization of cholesterol that leads to the formation of gallstones. The presence of such conditions, however need not ensure the occurrence of the nucleation and growth of cholesterol crystals. While it is generally agreed that the predominant driving force in the nucleation process is the absolute degree of cholesterol supersaturation, a number of other phenomena are known to be involved. Dilution markedly prolongs the nucleation time in supersaturated human and model bile (Gollish et al. 1983; Mazer and Carey 1983). The factors which lead to the formation of abnormal bile are the following:

* Losses of the bile salt in conditions during ileostomy, which is greater than hepatic synthetic capacity resulting in low bile salt secretion and subsequent cholesterol supersaturation.
Figure 1.2 An integrated model of gallstone formation.
* Inadequate bile acid synthesis increases the risk of cholesterol cholelithiasis, but is very uncommon and is found in the patients with cerebrotendinous xanthomatosis disease.

* Excessive cholesterol secretion which is found in the obese patients.

* Reduction in phospholipid synthesis.

* Presence of unmetabolized cholesterol.

* The dilute bile is less able to solubilize cholesterol than concentrated bile of the same relative lipid proportions.

Liver is mainly responsible for the production of abnormal bile, whilst the gallbladder is the reservoir that stores, concentrates and expels bile. Thus, it plays no significant role in the first stage (supersaturation) of gallstone pathogenesis, but does contribute to the next two stages. Many normal people will produce supersaturated hepatic bile during their overnight fast as fasting reduces bile salt secretion rates and increases the concentration of cholesterol accordingly. The supersaturated hepatic bile mixes with large amounts of unsaturated gallbladder bile in normal people to give an unsaturated mean composition.

1.6.4 Cholesterol crystal nucleation

It is common that heterogenous nucleation can occur because precipitation develops around the pre-existing substance such as mucus, calcium bilirubinate or bacterial fragments. Nucleation is more rapid in gallbladder bile for patients with cholesterol gallstones than normal persons. Hence the difference in nucleation period distinguishes the bile from
gallstone patients to that of normal persons. The difference can be explained by the presence of inhibiting or promoting factors in the bile.

Many people have more amount of cholesterol in bile, yet do not suffer from cholelithiasis. Sedaghat and Grundy (1980) showed that the ‘supersaturated’ bile in the people without gallstones do not contain cholesterol monohydrate crystals while patients with gallstones have cholesterol monohydrate crystals in gallbladder. Cholesterol saturation index seems to be an essential prerequisite for the formation of gallstone. This suggests that the bile of the gallstone patients lacks either proper inhibiting agent that normally prevents cholesterol crystallization or they have promoters for crystal nucleation. Studies by Whiting and Watts (1985) clearly showed that ‘supersaturated’ bile from obese subjects without gallstones would not form cholesterol crystals in vitro unless small seed crystals of cholesterol monohydrate were added. Another study by Burnstein et al (1983) indicated the presence of vital nucleating factor in the bile of people who have gallstones. It has been accepted that the bile supersaturated with cholesterol was a necessary and sufficient explanation of cholesterol precipitation and consequent gallstone formation. However, Holzbach et al (1973) reported that the supersaturated bile does not necessarily lead to the formation of gallstones. A few years later in attempting to explain the differences in the induction period in the case of abnormal and normal persons, it was introduced that qualitative differences in biliary proteins may be involved in the nucleation concept. From his study there is an evidence that biliary protein contains components capable of inhibiting cholesterol crystal nucleation in human gallbladder bile. And it has been shown that cholesterol crystals were found to form in the following situations:
* more slowly in supersaturated gallbladder bile from subjects without gallstones than in artificial bile solutions of identical lipid composition.

* more rapidly in supersaturated gallbladder bile from subjects without gallstones after removal of proteins than in the same bile before the removal of proteins.

They conclude that patients without gallstone disease have a cholesterol crystal nucleation inhibitor that is predominantly derived from bile proteins. This finding affords an explanation for the prolonged metastability found in normal human bile, especially in the presence of marked biliary cholesterol supersaturation. The precise mechanism whereby the inhibitory effect is mediated remains unclear and this may be of fundamental importance of both the definitions of health and the pathogenesis of cholesterol gallstone disease (Holzbach et al 1984; Kibe et al 1985; Whiting and Watts 1985).

Several substances normally present in bile were shown to be capable of altering the nucleation of cholesterol crystals. Among these substances, apolipoprotein A-1 (Kibe et al 1984) was found to inhibit crystallization whereas mucin promotes crystallization. The promoters of nucleation include mucoprotein and prostaglandin and inhibitors include lipoproteins and bile acids themselves (Gallinger et al 1985; Levy et al 1984).

### 1.6.5 Cholesterol gallstone growth

The microscopic crystals need time to grow to a macroscopic level and people without gallstones occasionally have been shown to have microliths in their bile. Sluggish gallbladder contraction and stratification
of the bile within the gallbladder influence gallstone growth. Pregnancy and diabetes mellitus have been associated with impaired gallbladder contraction. The concentration of ions such as calcium are thought to be important in determining the growth of crystal aggregates.

1.6.6 Pigment gallstones

There has been a lot of literature reports on the pigment gallstones (Burnett et al 1981; Busch and Holzbach 1990; Lee et al 1981; Soloway et al 1977; Whiting and Watts 1983). Pigment gallstones are stones that have a cholesterol content less than 20%. They are of two types: brown and black stones. Brown stones are also called 'calcium bilirubinate'. They occur all over the biliary ductal system and are almost associated with infection. They have a dull, earthy brown surface and are easily crushable. When they are viewed along the cross section, they appear laminated, with a characteristic pattern of alternating dark(brown) and light(tan) concentric layers. The brown layer is made of calcium bilirubinate and contains very little fatty acids, whereas the tan layer contains predominantly fatty acids, the major one being calcium palmitate. On scanning electron microscopy, the brown rings appear smooth and featureless, while the fatty acid rich tany layers are rough with the stacks of jagged plates. Foreign objects such as migrating cholesterol and black pigment stones, parasites and surgical suture material are associated with the formation of brown pigment stone. The black stones show much variation in the texture, colour and composition. They are divided into two sub-types depending on their composition: the carbonate and non-carbonate black pigment gallstones. The non-carbonate stones may be either black phosphate or black bilirubinate stones. The cut surface of the black carbonate stones show pits and granules. Layering is also seen. The narrow rough rings are rich in calcium and phosphorous while the wider and smoother rings are rich in sulphur. The non-carbonate stones have a smooth surface and a homogeneous cross
section. The black bilirubinate stones are most homogeneous. The phosphate stones have a rough surface at the centre which is rich in sulphur and copper. In general, the smooth areas in pigment calculi are associated with calcium bilirubinate and high concentration of sulphur.

The structural and compositional differences suggest that the pigment calculi is formed by varied mechanisms. While the brown stones are believed to be the outcome of infection, the black stones are products of some metabolic abnormality.

Hard mineral deposits in the initial nidus might injure the gallbladder mucosa and cause microhaemorrhage which might further promote nucleation. Similar events could presumably be occurring during pigment gallstone formation.

Although it is not clear which biles are supersaturated with calcium bilirubinate one can speculate the factors that promote the precipitation of calcium bilirubinate to form brown pigment stones.

1.7 Atherosclerosis

Atherosclerosis is associated with the focal accumulation in arteries by three major classes of lipids namely cholesterol, cholesteryl ester and phospholipids. At body temperature, these lipids can exist in three distinct physical states such as crystalline, liquid crystalline and liquid.

The lipid - lipid interactions have been expressed in the form of condensed phase diagrams and the compositional limits and structure of each phase have been determined by X-ray diffraction, calorimetry and polarizing microscopy. By plotting the lipid composition of intimal lesions at different stages of severity on a phase diagram (Small and Shipley 1974)
describing overall intimal lipid-lipid interactions, it is possible to predict the number and the physical state of the lipid phase that should be present in the lesions.

The phase diagram as shown in Fig. 1.3 is a four component system, phospholipid (PL), cholesterol ester (CE), cholesterol (C) and water at 37°C and one atmospheric pressure. The amounts of the components present are expressed as weight percentage. The tetrahedron (A) illustrates the classical representation of a four component system whereby each apex represents 100% of a single component, and any point within the tetrahedron represents a mixture of a specific composition. (B) For the purpose of illustrating the three component systems, the tetrahedron has been spread open from the water apex. On the right is the three component system (PL-C-Water).

Zone 1: A single phase, the lamellar liquid crystalline phase containing upto 33% by weight of C,

Zone 2: A two-phase zone consisting of hydrated lamellar liquid crystals of PL and C, and free water,

Zone 3: A two-phase zone containing liquid crystals of PL and C in equilibrium with crystals of C,

Zone 4: An invariant three phase zone consisting of liquid crystals of PL saturated with C and water, free water, and C crystals. At the bottom is the three component system, PL-CE-water,

Zone 5: A single phase of lamellar liquid crystal containing upto 2 to 3 percent CE,

Zone 6: A three phase zone of lamellar liquid crystal and free water,

Zone 7: A two phase zone, lamellar liquid crystal saturated with CE in equilibrium with CE,
Figure 1.3 Four component system of phospholipid (PL), cholesteryl ester (CE), cholesterol (C) and water.
Zone 8: an invariant three phase zone consisting of lamellar liquid crystals saturated with CE, PL and free water. At the left is the three component system, CE-C-water,

Zone 9: A two-phase zone consisting of an oily phase of CE containing up to 78 percent of C and free water,

Zone 10: An invariant three-phase zone containing an oily phase of CE saturated with C, cholesterol crystals and water.

Cardiovascular disease accounts for about one quarter of the worldwide deaths. In developed countries about half of the deaths are due to cardiovascular disease (principally coronary heart disease and stroke). Atherosclerosis is the most frequent and associated with alteration of the intima and subintima of the arteries and particularly the coronary arteries.

The first event in the formation of atherosclerotic plaque is the formation of 'fatty-streak' which is an aggregation of lipid rich macrophages and T-lymphocytes in the intima of the artery (Ross 1993). Lipid-rich macrophages, which are also called as foam cells, are produced from the fatty streaks and the phagocytic macrophages. These cells ingest the fatty streaks and accumulate these particles in the cytoplasm and will be connected to foam cells which are deposited in the intimal layer of the artery. The formation of foam cells is the primary event which is followed by the involvement of various components such as smooth muscle cells, endothelial cells, platelets, coagulation factors, various growth factors and regulatory proteins. Therefore the formation of the fatty streak is considered to be an important step in atherogenesis. Fatty-streak contains many lipids mainly cholesterol, cholesteryl esters and phospholipids. Observations in animal models have shown that fatty streaks precede the development of intermediate lesions, which are composed of layers of macrophages and smooth muscle cells and in turn, develop into the more advanced, complex and occlusive lesions called fibrous plaques. The fibrous plaques increase in
size and by projecting into the arterial lumen, may impede the flow of blood. The cellular events that occur during progression of lesions in hypercholesterolaemic animals are almost exactly mirrored those observed in atherosclerotic coronary arteries in hearts that are removed in transplant operations. Progression of atherosclerotic lesions is thus marked by the accumulation of alternating layers in the smooth muscle. The atherosclerotic process is slow and reflects continuous exposure to a variety of both identified and unidentified risk factors.

Lipid accumulation in atherosclerosis is associated with a slower net rate of efflux than influx of lipids in the vessel wall. Moreover, the presence of crystals and liquid crystals limits the enzyme accessibility into the substrate sites within the molecules. Thus, physical states of lipid would be of importance both in the formation and regression of plaques. Epidemiological studies have led to the recognition of as many as 246 risk factors (major, minor, reversible and irreversible). The risk factors are divided into a) non-modifiable, b) modifiable and c) miscellaneous. The first two are known as the primary risk factors and the third one is known as the secondary risk factor. Modifiable risk factors include hypertension, cigarette smoking, cholesterol, serum triglycerides, phospholipids, free fatty acids, diet, psychological and behavioural factors. The Non-modifiable risk factors include age, sex, family history or genetic predisposition. The miscellaneous risk factors include diabetes mellitus, obesity and oral contraceptives.

The several risk factors, in one way or the other, aggravate the atherosclerotic process and are responsible for the precipitation of symptomatic ischemic heart disease by influencing one or more of the following factors or ratios, which can be used as predictors and their changes with time and therapy used as a measure of the progression or regression of the atherosclerotic process;
a) Total/HDL cholesterol ratio, known as coronary risk index (CRI)
b) Increased low density lipoprotein cholesterol.
c) Decreased high density lipoprotein cholesterol.
d) Plasma cholesterol/phospholipid molar ratio (C/P ratio).

Cholesterol is a pivotal element in the aetiology of atherosclerosis. Many studies have confirmed that free and esterified cholesterol accumulates in the aorta, coronary arteries and cerebral vessels and the rate of accumulation varies among individuals (Zilversmit 1979).

Though there are a number of ways by which atherosclerosis (for example, coronary artery bypass surgery, coronary angioplasty) can be treated, the use of cholesterol lowering drugs are preferred, mostly during the early stages of the disease. The drugs in vogue are clofibrate, cholestyramine, gemfibrozil, lovastatin, simvastatin, pravastatin, niacin-colestipol combination and colestipol-lovastatin combination. Their efficacy in lowering the cholesterol level and thus the risk of coronary heart disease is well proved. The use of these drugs are not without any side effects. For example, deaths due to cancer, violent accidents and suicide are important concern over using clofibrate (Durrington 1992).

The drug lovastatin has been complained to disturb sleep (Schaefer 1988) and to affect daytime performance (Richardson et al 1991; Thomson 1992). Changes in daytime performance have been observed on patients administrating lovastatin, pravastatin and placebo. Also it has been questioned whether the use of such cholesterol lowering drugs would increase non-cardiac mortality since it is doubted to alter the composition of cell membranes and thus enhanced predisposition to accidental death (Oliver 1991). In this context it is to be mentioned that the medicinal plants used in the present study interfere only with the crystallization behaviour.
of cholesterol at a given concentration, however *in vivo* studies are warranted to rule out any possible membrane damages.

Diet has been playing a significant role in the atherosclerosis, since dietary cholesterol is taken up by the body completely, the increased uptake reflects in plasma cholesterol levels. To make a lipid lowering therapy successful, an appropriate dietary therapy is essential. Also an increased intake of high fat diet results in obesity which increases cardiac work load (Durrington 1992). Evidences suggest that for the children, the level of blood cholesterol and low density lipoprotein are found to be lowered after diet or drug treatment (West and Fosbrooke 1975).

Consumption of marine oily fish or oils from these fishes/marine mammals play an important role in reducing the risk of CHD. However they don't have the cholesterol lowering effect in general. But lowering of triglyceride level has been shown to be the reason for the reduced risk (Harris 1989).