Atherosclerosis comes from the Greek words ‘athero’ (meaning gruel or paste) and ‘sclerosis’ (hardness), and refers to the process in which deposits of fatty substances, cholesterol, cellular waste products, calcium and other substances build up in the inner lining of an artery, leading to plaque formation. It is a chronic inflammatory disorder driven by risk factors that cause oxidative inflammatory mechanisms; an important underlying pathology of CVD, the leading cause of morbidity and mortality in developed countries (Libby, 2002).

1. Process of atherogenesis

The majority of CVD results from complications of atherosclerosis. An important initiating event for atherosclerosis may well be the transport of Ox-LDL across the endothelium into the artery wall (Navab et al., 1996). This is likely to occur at the sites of endothelial damage which are caused by Ox-LDL itself, as well as physical or chemical forces and infection (Fig. 1) (Ross, 1999). Endothelial cells, smooth muscle cells (SMCs) and macrophages are the sources of oxidants for the oxidative modification of phospholipids. Ox-LDL can damage endothelial cells and induce the expression of adhesion molecules such as P-selectin (Vora et al., 1997) and chemotactic factors such as monocyte chemoattractant protein-1 (MCP-1) and macrophage colony-stimulating factor (mCSF) (Cushing et al., 1990; Rajavashisth et al., 1990). These processes lead to the tethering, activation and attachment of monocytes and T lymphocytes to the endothelial cells (McEver, 1992). Endothelial cells, leukocytes, and SMCs then secrete growth factors and chemo-attractants which effect the migration of monocytes and leukocytes into the sub-endothelial space (Ross, 1993). Monocytes ingest lipoproteins and transform into macrophages; macrophages generate ROS, which convert Ox-LDL into highly oxidized LDL, which is, in turn, taken up by macrophages to form “foam cells”. Foam cells combine with leukocytes to become the fatty streak; as the process continues, foam cells secrete growth factors that
induce migration of SMCs into the intima. Proliferation of SMCs coupled with the continuous influx and propagation of monocytes and macrophages, converts fatty streaks to more advanced lesions. Ultimately, a fibrous plaque forms that protrudes into the arterial lumen. Later, calcification may occur and fibrosis may continue, yielding a fibrous cap that surrounds a lipid-rich core. This formation may also contain dead or dying SMCs. In acute coronary syndromes (e.g. myocardial infarction), the fibrous plaques rupture, leading to the formation and release of thrombi that may ultimately occlude vessels (Fig. 1).

2. Risk factors associated with atherosclerosis

The development of atherosclerosis is governed by various risk factors such as hyperlipidemia, hypertension, obesity, smoking, sex and age (Scott, 2004). Such risk factors may be classified as

1. Modifiable
2. Non-modifiable
3. Miscellaneous
Modifiable and non-modifiable risk factors are also known as primary risk factors, whereas miscellaneous risk factors are known as secondary risk factors (Table 1) (Hopkins and Williams, 1981).

Table 1. Risk factors for atherosclerosis

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Primary pathogenesis</th>
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<tbody>
<tr>
<td>1. Modifiable risk factors</td>
<td></td>
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<tr>
<td>Smoking</td>
<td>Oxidative stress</td>
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<tr>
<td>Atherogenic diet</td>
<td>High blood cholesterol and oxidative stress</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Oxidative stress and enhanced vasoconstriction</td>
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<tr>
<td>2. Non-modifiable risk factors</td>
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<tr>
<td>Age and gender</td>
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<tr>
<td>Family history of premature CHD</td>
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<td>Ethnicity</td>
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<tr>
<td>3. Miscellaneous risk factors</td>
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<tr>
<td>Physical inactivity</td>
<td>Poor perfusion and adverse lipid profile</td>
</tr>
<tr>
<td>Obesity</td>
<td>Metabolic syndrome of resistance to insulin</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>a. Infection</td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus, Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus</td>
<td>Inflammation</td>
</tr>
<tr>
<td>b. New and emerging risk factors</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
<td>Thrombogenesis</td>
</tr>
<tr>
<td>CRP</td>
<td>Inflammation</td>
</tr>
<tr>
<td>3. Pathogenesis of atherosclerosis</td>
<td></td>
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Atherosclerotic lesions or atheromata are asymmetric focal thickenings of the innermost layer of the artery, the intima. They consist of cells, connective-tissue elements, lipids, and debris (Stary et al., 1995). Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular endothelial and SMCs. The atheroma is preceded by a fatty streak, an accumulation of lipid-laden cells beneath the endothelium (Stary et al., 1994). Most of the cells in the fatty streak are macrophages, together with some T lymphocytes. Studies in animals
and humans have shown that hypercholesterolemia causes focal activation of endothelial cells in large and medium-sized arteries.

3.1. Hypercholesterolemia

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and subsequent CVD (Steinberg, 2002). Exogenous hypercholesterolemia causes deposition of fat in the liver and depletion of the hepatocyte population; it can also cause malfunctioning of the liver, which apparently follows microvesicular steatosis due to the intracellular accumulation of lipids (Gupta et al., 1976; Assy et al., 2000). Feeding animals with excessive amounts of cholesterol has often been used to elevate serum or tissue cholesterol levels in order to study the etiology of hypercholesterolemia-related metabolic disturbances (Bocan, 1998). Hypercholesterolemia-induced microvascular alterations can be demonstrated in animal models within a few days after feeding a diet enriched with cholesterol, i.e., long before the appearance of fatty streak lesions in large arteries (Scalia et al., 1998; Stokes et al., 2001). The extent of hepatic damage can be assessed by noting the mean serum activities of hepatic marker enzymes such as transaminases and alkaline phosphatase (Molander et al., 1955). The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were found to be elevated in an experimental hypercholesterolemic rat model (Sudhahar et al., 2007).

Atherosclerosis, the primary cause of heart disease, is characterized by increasing amounts of free cholesterol (FC) and esterified cholesterol (EC), which accumulate in foam cells derived from both macrophages and SMCs (Jerome and Lewis, 1987). The free cholesterol is then translocated to the cell membrane for its efflux and this transport of cholesterol from cells to the liver for excretion is known as reverse cholesterol transport (Rothblat et al., 1992). ACAT is a membrane-associated enzyme which is primarily localized in the endoplasmic reticulum. Its localization in the endoplasmic reticulum is consistent with its demonstrated role in cholesterol
transport within the liver and intestinal mucosa. ACAT is primarily responsible for the esterification of cholesterol in all mammalian cells; it has also been implicated in intestinal mucosal absorption of cholesterol, and in the synthesis of the cholesterol esters which are incorporated into VLDL or stored in fatty cells (Krause et al., 1993; 1995). High-density lipoproteins are thought to play an important role in this process, and their ability to stimulate removal of cholesterol from peripheral cells may explain their negative association with CVD (Williams et al., 1979). ACAT appears to be involved in regulation of hepatic cholesterol 7α-hydroxylase, the rate-controlling enzyme in cholesterol catabolism (Post et al., 1999). The 7α-hydroxylase enzyme is presumed to play a crucial role in foam cell formation from macrophages and vascular SMCs in the arterial wall in the pathophysiological state (Son et al., 2003). Under pathological conditions, accumulation of ACAT reaction-products, such as foamy lipid droplets in the cytoplasm of macrophages and SMCs, is a characteristic feature of early lesions of atherosclerotic plaque (Brown and Goldstein, 1983); other workers have shown that over-expression of hepatic ACAT might also be involved in the pathogenesis of hypercholesterolemia (Nosratola and Liang, 2002).

Homeostasis of cellular cholesterol is maintained by regulation of its de novo synthesis, uptake from plasma and formation of bile acid in the liver. Catabolism of cholesterol to bile acids is an important route for its elimination from the body and accounts for about 50% of the daily elimination of cholesterol. A high-cholesterol diet is known to induce the rate-limiting enzyme for synthesis of bile acids namely cholesterol 7α-hydroxylase, which, in turn, stimulates the conversion of cholesterol to bile acids; these events facilitate disposal of excess cholesterol (Jones et al., 1993).

Fatty acids play a key role in the formation and maintenance of all cells. Phospholipids are the major structural components of plasma and intracellular membranes in all living organisms. Dietary fatty acids are also known to modulate the lipid composition of biological membranes and their fluidity (Stubbs and Smith, 1984). Experimental evidence suggests that a diet rich in saturated fatty acids (SFA) is
associated with high levels of serum cholesterol which, in turn, is related to a high incidence of CHD (Parthasarathy et al., 1990b). The putative atherogenic SFA are C12:0 (lauric), C14:0 (myristic) and C16:0 (palmitic). Hegsted et al. (1965) suggested that myristic acid was the most atherogenic, with about four times the cholesterol-raising potential of palmitic acid. Dietary trans-fatty acids are formed when liquid vegetable oils are heated in the presence of meal catalysts (Ascherio and Willett, 1995). Trans-fatty acids extend the effects of saturated fat and cholesterol on blood lipids, that is, they increase the LDL cholesterol and reduce HDL cholesterol. Both epidemiological and clinical studies support these adverse effects of partially-hydrogenated vegetable oils on risk of CAD (Mensink and Katan, 1990).

3.2. Lipoproteins and atherosclerosis

Serum cholesterol is carried by several lipoprotein particles that perform the complex physiologic task of transporting dietary and endogenously produced lipids (Witztum and Steinberg, 1995). In the intestine, dietary triglycerides and cholesterol are absorbed into the lymphatics and are incorporated into chylomicrons. The chylomicrons then enter the blood at the thoracic duct and most of the triglycerides are hydrolysed by lipoprotein lipase (LPL), an enzyme bound to the external surface of the capillary endothelium. The free fatty acids, which are the products of hydrolysis, enter either the adjacent muscles where they are used for energy, or the adipose cells where they are used for resynthesis of triglycerides for storage of energy (Brown et al., 1981). During the process, the chylomicron particle loses about 96% of its mass of triglycerides and, finally, the apolipoproteins A and C are transferred to the HDL in circulation (Cooper, 1985).

Dietary cholesterol derived from the receptor-mediated uptake of chylomicron remnants is insufficient, hence, the liver synthesises its own cholesterol by increasing the activity of the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA-reductase) (Brown et al., 1981). The endogenously-made triglycerides and cholesterol are then packed into VLDL, which enters the systemic
circulation (Havel, 1994). VLDL triglycerides are hydrolysed at the endothelial cell surface by lipoprotein lipase to produce smaller, more cholesterol-enriched particles, which are then metabolized to intermediate density lipoprotein (IDL). Liver clears the VLDL remnants and the IDL is ultimately converted into LDL (Grundy, 1986). LDL, a major cholesterol-carrying lipoprotein fraction in human blood plasma, is known to be a risk factor for the development of atherosclerosis (Steinberg et al., 1989) and has also been shown to accumulate within the arterial wall. It is well-established that increased levels of LDL cause an increased risk for atherosclerosis (Goldstein and Brown, 1977).

3.3. Oxidative stress and atherosclerosis

Oxidative stress can be defined as a state resulting from disruption of the delicate balance between oxidative (pro-oxidative) and antioxidative processes. Oxidative stress is postulated to play an important role in the pathogenesis of hypercholesterolemic atherogenesis (Steinberg et al., 1989). Feeding a cholesterol-rich diet to rabbits produces severe hypercholesterolemic and vascular atherosclerotic lesions and increased oxidative stress in several tissues (Balkan et al., 2002). Thus, there is clear evidence that oxidative stress contributes to the development of atherosclerosis in the vascular wall through the formation of ROS (Shi et al., 2000). Modification of LDL via oxidative processes is believed to be a prerequisite for the development of atherosclerosis (Hazen, 2000). The ‘oxidative modification hypothesis’ of atherosclerosis proposes that oxidation of LDL cholesterol is an early event in atherosclerosis and that Ox-LDL contributes to atherogenesis; lipid peroxidation occurs in arterial macrophages as well as in lipoproteins (Aviram, 2000).

According to the oxidative-modification hypothesis, LDL initially accumulates in the extracellular sub-endothelial space of arteries and, through the action of resident vascular cells, LDL is mildly oxidized to a form known as minimally modified LDL (Navab et al., 1996). This minimally modified LDL induces local vascular cells to produce MCP-1 and granulocyte and macrophage colony-stimulating factors, which stimulate monocyte recruitment and differentiation to macrophages in arterial walls.
(Parhami et al., 1993). The accumulating monocytes and macrophages stimulate further peroxidation of LDL. The products of this reaction make the protein component of LDL (apolipoprotein B-100) more negatively charged. By virtue of its increased negative charge, this completely oxidized LDL is recognized by scavenger receptors on macrophages and internalized to form the foam cells (Henriksen et al., 1981). In contrast to the uptake of native (un-oxidized) LDL by the LDL (apolipoproteins B and E) receptor on macrophages, the uptake of Ox-LDL by the scavenger receptor pathway is not subject to negative-feedback regulation and thus results in massive uptake of cholesterol by the macrophages. In addition to promoting the formation of foam cells, Ox-LDL has direct chemotactic activity for monocytes (Quinn et al., 1988) and stimulates the binding of monocytes to the endothelium (Frostegard et al., 1991). Once monocytes cross the endothelial layer, they become trapped in the sub-endothelial space, because Ox-LDL inhibits their egress from the arterial wall (Quinn et al., 1987). Ox-LDL is also cytotoxic to vascular cells (Cathcart et al., 1985; Schwartz et al., 1991), thus promoting the release of lipids and lysosomal enzymes into the intimal extracellular space and enhancing the progression of atherosclerotic lesions (Schwartz et al., 1991). The oxidative-modification hypothesis is supported by evidence that oxidation of LDL occurs in vivo and contributes to the clinical manifestations of atherosclerosis. Thus, oxidative modification of LDL appears to have an important role in foam cell formation and atherogenesis. Erythrocytes are perforce in an environment in which they are constantly exposed to both extracellular and intracellular sources of ROS. Hypercholesterolemia leads to increased accumulation of cholesterol in the erythrocytes and endothelial cells, thereby activating and enhancing them to produce oxygen free radicals (Prasad and Kalra, 1989; Kay, 1991). Therefore, erythrocytes are extremely vulnerable to these antioxidant challenges and hypercholesterolemia. Akkus et al. (1996) reported that the antioxidant status of erythrocytes is altered in CVD.

3.4. Inflammatory reaction and atherosclerosis

Atherosclerosis is also considered to be a chronic inflammatory disease, since it develops in response to damage to the vessel wall. It is characterized by the infiltration
of mononuclear blood cells into the intima, formation of ‘foam cells’, the proliferation of arterial smooth muscle cells and accumulation of connective tissue components in the inner lining of the arteries (Ross, 1999; Glass and Witztum, 2001). CRP is one of the substances present in the atherosclerotic lesion, more specifically in the vascular intima, where it co-localizes with monocytes, monocyte-derived macrophages and lipoproteins (Torzewski et al., 1998; Zwaka et al., 2001). Two hypotheses have been proposed to explain the significance of an elevated serum CRP concentration in the pathogenesis of atherosclerosis. One mechanism may be the ongoing inflammation in the artery, stimulated by Ox-LDL, which leads to the production of cytokines that induce the formation of CRP, an acute phase protein. Alternatively, chronic elevations of acute phase reactants could be due to smoking, chronic infections, obesity and hypercholesterolemia, all of which contribute to the development of atherosclerosis. However, these hypotheses are not exclusive and both mechanisms might act together to account for the increase of CRP (Francisco et al., 2006).

CRP is primarily synthesized in hepatocytes, although a growing body of evidence indicates extrahepatic production of CRP, such as in macrophages, arterial tissue, adipose tissue or endothelial cells (Dong and Wright, 1996; Yasojima et al., 2001; Ouchi et al., 2003; Venugopal et al., 2005). The expression of mRNA coding for CRP has also been identified in many different extrahepatic cells, such as islet cells of the pancreas (Fehsel et al., 1997), neurons (Yasojima et al., 2000), adipocytes (Ouchi et al., 2003) and renal tubular epithelial cells (Jabs et al., 2003). The elevated level of CRP in blood constitutes a stable and reliable marker of systemic inflammation (Gabay and Kushner, 1999; Roberts et al., 2001; Rothkrantz-Kos et al., 2002; Prasad, 2003). The erythrocyte sedimentation rate (ESR) is also frequently used as an indicator of inflammation and as an independent predictor of CVD (Danesh et al., 2000; Godsland et al., 2004).

Feeding a cholesterol-rich diet induces free radical production, followed by oxidative stress and hypercholesterolemia (Bulur et al., 1995) in rats, and also induces
signs of inflammation, such as migration of white cells into the endothelium and expression of adhesion molecules in rabbits (Li et al., 1993). Various inflammatory cells, including macrophages and lymphocytes, are able to generate ROS (Russwurm et al., 1994). Enhanced oxidative stress due to the inflammatory response might thus contribute to the pathogenesis of atherosclerosis. The systemic response to inflammation increases the level of oxidized lipids in serum and enhances the oxidative modification of LDL-cholesterol (Memon et al., 2000). In addition, CRP has a pro-oxidative effect (Kobayashi et al., 2003). Currently both oxidative and inflammatory processes are believed to be involved in the pathogenesis of atherosclerosis (Hansson, 2005), where antioxidant defence systems are also impaired.

3.5. Apoptosis and atherosclerosis

Apoptosis is a physiologic process that is important in the normal development and homeostasis of multicellular organisms (Raff, 1992). Derangement of apoptosis can have deleterious consequences as exemplified by several human diseases, including acquired immunodeficiency syndrome, neurodegenerative disorders, cancer and atherosclerosis. Several authors have reported evidence for the occurrence of apoptosis in human atherogenesis and in experimental models (Isner et al., 1995; Bochanton Piallat et al., 1995; Bennett et al., 1995; Rembold, 1996). Apoptosis of vascular cells may be involved in the progression of atherosclerosis (Han et al., 1995; Bjorkerud and Bjorkerud, 1995; Irani, 2000). Apoptotic endothelial cells have been reported to contribute to endothelial dysfunction and destabilization of atherosclerotic plaques and thrombosis (Asai et al., 2000; Chen et al., 2005), suggesting that apoptosis of endothelial cells plays an important role in initiation and progression of atherosclerosis. A wide variety of stimuli, including oxidative stress, can induce apoptosis of endothelial cells and endothelial cell dysfunction, which may be regulated by different signal pathways (de Nigris et al., 2003). There is evidence to show that ROS can activate c-Jun NH2-terminal kinase (JNK), p38 and mitogen-activated protein kinases (MAPK); this activation triggers subsequent apoptosis such as the activation of caspase proteases in certain cells (Jiang et al., 2004; Osone et al., 2004; Pearl-Yafe et al.,
Caspases, a family of cysteine proteases, play a pivotal role in the process of apoptosis. There are two independent pathways by which apical caspases may be activated, and these involve activation of receptor-intermediate caspase-8 and mitochondria-cytochrome-c-intermediate caspase-9. Both caspase-8 and caspase-9 activate the executioner, caspase-3, which, in turn, is responsible for the terminal phase of apoptosis (Nunez et al., 1998). On activation of the protease cascade, the caspase-3 pro-enzyme is proteolytically cleaved into p20 and p11 subunits which are then heterodimerized to form the active enzyme (Fernandes-Alnemri et al., 1994).

Induction of caspase-3 proteolytic activity appears to be one of the most important events in apoptosis (Harvey et al., 1998; Sakahira et al., 1998). Caspase-3-mediated apoptosis may be modulated by cellular oxidative stress. The direct involvement of oxidative stress in apoptosis has been demonstrated in a variety of cell types, such as aortic endothelial and smooth muscle cells (Hockenbery et al., 1993). Increased formation of oxygen radicals facilitates LDL oxidation and influences oxidation-sensitive mechanisms (Napoli, et al., 2001). Ox-LDL has been shown to induce apoptosis in numerous cell types, including ECs, SMCs, macrophages and lymphoid cells (Mallat and Tedgui, 2000; Auge et al., 2000). An increase in caspase-3-like protease activity in human endothelial cells has been found associated with the induction of apoptosis by Ox-LDL (Dimmeler et al., 1997). Exposure of endothelial cells to interleukin-4 (IL-4) can also increase cellular oxidation (Lee et al., 2000); it is possible that caspase-3 may be activated by this cytokine. Thus, there is evidence that apoptosis of endothelial cells contributes to the initiation of atherogenesis.

4. **Antioxidant defense system**

The oxidation induced by ROS can result in disintegration of cell membranes, damage to membrane protein and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and CVD (Liao and Yin, 2000). Oxidative stress, which results from impairment of the equilibrium between production of free radicals and antioxidant defense systems, is one
of the factors that links hypercholesterolemia with atherogenesis (Halliwell, 1996). There is evidence that oxidative stress contributes to the development of atherosclerosis in the vascular wall through the formation of ROS (Shi et al., 2000). In order to protect the tissues from damage caused by ROS, organisms possess enzymatic and non-enzymatic antioxidant systems (Parthasarathy et al., 2000). Protection against ROS and the breakdown products of peroxidized lipids and oxidized proteins is provided by enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and glutathione-S-transferase (GST). Non-enzymatic antioxidants, such as reduced glutathione (GSH), vitamin C and vitamin E, play a vital role in protecting cells from oxidative stress by participating in various biochemical pathways.

Clinical trials suggest that vitamin E reduces platelet aggregation, and the strong association between low serum selenium and platelet aggregation indicates that antioxidants may be important in the regulation of platelet function (Salonen, 1989). Abundant data from epidemiologic observational studies suggest that greater intake of antioxidant vitamins such as vitamin E, vitamin C, and beta carotene are associated with reduced risk of CVD (Rimm and Stampfer, 2000). A number of animal studies have also demonstrated that the consumption of polyphenols limits the development of atherosclerotic lesions. Supplementation of drinking water with dealcoholized wine, pomegranate juice and quercetin was found to reduce the size of these lesions in apolipoprotein E-deficient mice (Hayek et al., 1997; Kaplan et al., 2001). These effects are associated with reduced uptake of LDL cholesterol by macrophages, and decreased susceptibility of LDL to aggregation. Grape seed polyphenols, proanthocyanidins, have a hypocholesterolemic effect on rats fed a high-cholesterol diet (Tebib et al., 1994). Piperine, the major active principle of black pepper, has been reported to show a wide range of pharmacological properties such as antioxidant (Mittal and Gupta, 2000), hepatoprotective (Koul and Kapil, 1993), anti-inflammatory (Mujumdar et al., 1990), immunomodulatory (Sunila and Kuttan, 2004), anti-tumourigenic (Nalini et al., 2006) and anti-hyperlipidemic (Vijayakumar and Nalini, 2006a) activities. Somova et al. (2003) reported that the naturally-occurring triterpenes, oleanolic acid and ursolic acid,
possess anti-hyperlipidemic (anti-atherosclerotic) and antioxidant activities. Lupeol, also a triterpene, exhibits anti-inflammatory (Geetha and Varalakshmi, 1998) and anti-hyperlipidemic (Sudhahar et al., 2006a) effects in experimental rat models. Ellagic acid, the phenolic compound present in fruits and nuts, including blueberries, blackberries, raspberries, strawberries and walnuts (Anderson et al., 2001; Sellappan et al., 2002; Talcott and Lee, 2002), has been found to have antimutagenic (Barch et al., 1996) and antioxidative (Priyadarsini et al., 2002) properties. However, studies have also shown that a synthesized novel antioxidant compound, N-(4,6-dimethyl-1-pentylindolin-7-yl)-2,2-dimethylpropanamide, can inhibit ACAT activity and also simultaneously inhibit LDL oxidation and cholesterol esterification (Kamiya et al., 2000; Nakamura et al., 2004).

5. Treatment of atherosclerosis

According to the National Cholesterol Education Program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (2001; 2002), the classical risk factors for CVD are commonly associated with hyperlipidemia and low concentrations of HDL-cholesterol (Knopp, 1999). There is comprehensive evidence that prevention and treatment of CVD optimally consists of reducing risk factors, such as changes in lifestyle, along with specific treatment of lipid disorders (Grundy et al., 2004). Meta-analyses from several primary and secondary interventional studies have clearly shown that cholesterol-lowering medication, mainly HMG CoA-reductase inhibitors, statins (atorvastatin, lovastatin, simavastatin and pravastatin) and fibrates, significantly reduces cardiovascular events, mortality, and morbidity (LaRosa et al., 1999; Ross et al., 1999; Bucher et al., 1999; Auer et al., 2001; Kreisberg and Oberman, 2002). Whereas fibrates and nicotinic acid cause significantly less lowering of LDL-cholesterol than statins, treatment with gemfibrozil and nicotinic acid produces a stronger reduction in triglyceride levels and more efficiently increases the level of HDL-cholesterol (Streja, 2004), which may be of equal importance for the prevention of CVD. Nicotinic acid is a highly potent drug for increasing HDL cholesterol levels up to 35% in patients with CVD (Chapman et al., 2004).
and further reduces LDL-cholesterol and triglycerides (Rader, 2003). All statins lower triglyceride levels up to 20-30%, and therefore, they are also useful in the treatment of moderate hypertriglyceridemia. Besides their lipid-lowering activity, statins are able to inhibit progression of atherosclerosis and to stabilize atherosclerotic plaques (Libby and Aikawa, 2002). However, cholesterol-lowering effects in prevention of CHD should not be solely orientated to statins and fibrate therapy, but may also include other hypolipidemic drugs, including the recently-introduced selective inhibitor of cholesterol absorption, ezetimibe. This novel drug prevents the absorption of dietary and biliary cholesterol without affecting the absorption of triglycerides or fat-soluble vitamins. Based on the clinical trials, ezetimibe seems to have lesser side-effects than statins (Bays et al., 2001; Knopp et al., 2003). Resins such as cholestyramine, colestipol, and colesevelam hydrochloride decrease LDL-cholesterol levels up to 20% (Steinmetz, 2002), by binding bile acids in the intestine and thereby interrupting enterohepatic circulation, leading to increased hepatic conversion of cholesterol to bile (Shepherd et al., 1980).

Epidemiological studies have demonstrated that a diet high in antioxidant vitamins such as vitamin C and vitamin E are associated with lower cardiovascular morbidity and mortality (Diaz et al., 1997). Antioxidant therapy is believed to be effective in the early stages of atherosclerosis by preventing oxidation of LDL and the oxidative lesions of the endothelium (Kaliora et al., 2006). Observational studies have suggested an inverse association between the consumption of fresh fruits and vegetables, which are important sources of antioxidants, and the risk of CVD (Diplock et al., 1998; Gaziano, 2000; Visioli, 2000). Additionally, these antioxidant compounds may be involved in protection against CVD (Silva et al., 2004).

Natural antioxidant components

Recently, there has been renewed interest in finding naturally-occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Sasaki et al.,
The antioxidative phytochemicals, especially phenolic compounds found in vegetables, fruits and medicinal plants, are examples of such naturally-occurring antioxidants (Cai et al., 2004). Many medicinal plants contain large amounts of antioxidants, such as polyphenol, which play an important role in abstracting and neutralizing free radicals, quenching singlet and triplet oxygen, or in decomposing peroxides (Anderson et al., 2001). Major antioxidant compounds, such as ascorbic acid, vitamin E and phenolic compounds, occur naturally in vegetables, fruits, grains and pulses, and are potentially useful in reducing oxidative damage. Research to identify antioxidant compounds has thus become an absolute necessity.

The antioxidant potential of various legume seeds that contain polyphenolic constituents has been reported (Shahidi et al., 2001; Siddhuraju and Becker, 2003; Lou et al., 2004; Lou et al., 2004; Nilsson et al., 2004). Legume seeds also provide micronutrients, vitamins, carotenoids and phenolic compounds, all of which are considered to be bioactive compounds (Adsule and Kadam, 1989; De Pascual Teresa et al., 2000; Duenas et al., 2002, 2004). Lipid peroxides can be effectively quenched by plant phenolics (Periera de Silva et al., 2000; Czinner et al., 2001; Lodovici et al., 2001) and phenolic acids are known to be scavengers of various ROS (Morton et al., 2000). Dietary phenolic compounds exert a variety of biological actions such as scavenging of free-radical, chelation of metals and modulation of enzymatic activity; they have also recently been found to affect signal transduction, activation of transcription factors and gene expression (Srinivasan et al., 2005).

Carotenoids are reported to be potent antioxidants and scavengers of free radicals (Grassman et al., 2002). β-carotene is the major carotenoid in carrot and sweet potato (Burns et al., 2003). Ascorbic acid is reported to interact directly with radicals such as O_2^- and OH^· in plasma, thus preventing damage to red cell membranes (Beyer, 1994). Ascorbic acid is also reported to scavenge superoxide and hydroxyl radicals, and to regenerate α-tocopherol (Davey et al., 2000). α-tocopherol, which is one of the most widely-distributed, naturally-occurring antioxidants in the biological system, protects
against lipid peroxidation through its chain-breaking antioxidant action (Burton and Ingold, 1981). The reaction predominantly responsible for the antioxidant activity of tocopherol is the donation of hydrogen atoms, which results in the formation of tocopheroxyl radical (Lampi et al., 2002). Ascorbic acid and \( \alpha \)-tocopherol have been identified in the leaves of *Carchorus olitorious* L. (Azuma et al., 1999). Leaves of tea (*Camellia sinensis*) are also reported to contain various antioxidants such as ascorbic acid, \( \alpha \)-tocopherol and tea catechins (Goto et al., 1996; Yamamoto et al., 1996). Flavonoids and their related compounds have been reported to inhibit lipid peroxidation (Ratty and Das, 1998), to scavenge free radicals and active oxygen moieties (Hanasaki et al., 1994), to chelate ferrous ions (Morel et al., 1994) and to scavenge ROS that are generated enzymatically and non-enzymatically in cell-free systems (Chen et al., 1990; Cimanga et al., 2001). Catechins, which are tea flavonols, are well-known representatives of natural polyphenolic antioxidants (Graham, 1992; Rice-Evans et al., 1996).

6. Green tea and EGCG

Tea is one of the most popular beverages consumed worldwide, next only to water (Vinson, 2000; Cheng, 2004). It can be categorized into three types, depending on the level of fermentation, i.e., green (unfermented), oolong (partially fermented) and black (fermented) tea. In general, green tea has been found to be superior to black tea in terms of antioxidant activity, owing to the higher content of epigallocatechin, epigallocatechin gallate. Catechins, which are tea flavonols, are well-known natural polyphenolic antioxidants (Graham, 1992; Rice-Evans et al., 1996) and approximately 80% of green tea consists of flavonoids. Interestingly, the use of tea extracts as dietary supplements arises from the perception that some tea compounds have beneficial protective effects against chronic diseases (Chen et al., 2008). The presence of polyphenols in tea may contribute to its antioxidant effect by inhibiting ROS-generating enzymes (Stangl et al., 2007). Health benefits of tea catechins are often linked to their antioxidant activities, including scavenging of reactive oxygen and
nitrogen species, inhibition of transcriptional factors such as activator protein 1, and inhibition of oxidative enzymes such as lipoxygenase and cyclooxygenase (Higdon and Frei, 2003). The dietary intake of phenolic compounds in green tea (Vinson et al., 2004), red wine (Frankel, 1995), and olive oil (Aviram and Kassem, 1993) could inhibit oxidation of LDL and thereby reduce risk factors for CVD.

The natural product, EGCG, is the major polyphenolic constituent found in green tea, *Camellia sinensis* L (Demeule et al., 2002; Bettuzzi et al., 2006). Green tea contains many biologically active polyphenolic flavonoids, commonly known as catechins, which include EC, ECG, EGC and EGCG. EGCG is known to inhibit a vast array of biomedically relevant molecular targets and disease-related cellular processes (Doss et al., 2005; Khan et al., 2006). EGCG, the principal constituent of green tea (Giakoustidis et al., 2006), has been characterized as an antioxidant (Xu et al., 2004) with antitumorigenic (Mukhtar and Ahmad, 2000), and antiangiogenic properties (Cao and Cao, 1999). Administration of EGCG has been reported to ameliorate various pathological states, including cerebral ischemia (Simonyi et al., 2005), cancer (Lin et al., 1999; Beltz et al., 2006) Parkinson’s disease (Nie et al., 2002; Guo et al., 2005; 2007) and Alzheimer’s disease (Bastianetto et al., 2006; Reznichenko et al., 2006; Avramovich-Tirosh et al., 2007). EGCG has also been found beneficial in treating obesity (Klaus et al., 2005; Kao et al., 2006c; Moon et al., 2007) and diabetes (Waltner-Law et al., 2002; Kao et al., 2006c; Wolfram et al., 2006).

7. **In vitro antioxidant assays**

The antioxidant potential of a compound can be attributed to its various characteristics, the most important of these being the ability to chelate transition metal ion catalysts (Rajeshwar et al., 2005) and to quench singlet-excited fluorescence of 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) (Nau, 1998). The processes governing the initial formation of radicals to initiate the radical chain reaction or radical-mediated lipid peroxidation must be catalyzed (Nawar, 1996). Transition metals are believed to serve as the catalysts for the initial formation of radicals. Chelating agents may stabilize
transition metals in living systems and inhibit generation of radicals, consequently reducing free radical-induced damage. The extract of *Chrysanthemum morifolium* was found to exhibit significant metal-chelating activity by reducing the concentration of the catalyzing transition metal during lipid peroxidation (Duh *et al.*, 1999). Extracts of *Vigna aconitifolia* (Siddhuraju, 2006), *Coleus aromaticus* (Kumaran and Karunakaran, 2006) and *Mentha spicata* (Kanatt *et al.*, 2007) have been reported to exhibit metal-chelating activity. Interestingly, the property of chelating cupric ions has been extensively studied in twenty-five plant extracts (Wong *et al.*, 2006). Methanolic extracts of medicinal mushrooms, such as *Ganoderma lucidum*, *G. lucidum antler*, *G. tsugae* and *Cariolus vercicolor*, have been found to chelate ferrous ions (Mau *et al.*, 2002). Ethanolic extracts of the oyster mushroom, *Pleurotus ostreatus*, and of the leaves of *Elaeocarpus ganitrus*, have been reported to chelate ferrous ions (Sathish Kumar *et al.*, 2008; Jayakumar *et al.*, 2009). Since ferrous ions are considered to be the most important pro-oxidants in the food system, the higher the chelating activity of a compound/extract, the greater its beneficial effect (Yamaguchi *et al.*, 1988).

"Fluorescence quenching" refers to any process which decreases the intensity of fluorescence of a particular substance. It has been widely studied both as a fundamental phenomenon and in the application of fluorescence to biochemical problems (Lakowicz, 1983). The fluorophore, DBO, has an extremely long fluorescence lifetime (up to 1 μs) (Nau, 1998). Its n,π*-excited states behave in a radical-like way. Since the reactivity resembles simple alkoxy radicals (Valgimigli *et al.*, 1995), it has been extensively employed as a fluorescent probe for antioxidants (Gramlich *et al.*, 2004; Pischel *et al.*, 2006; Jayakumar *et al.*, 2009). The quenching activity of other antioxidants, such as glutathione, uric acid and α-tocopherol, have been reported by Nau (1998), while Pischel *et al.* (2006) described the fluorescence quenching of singlet-excited DBO by 22 phenols and 12 alkyl benzenes.
8. Animal model of atherosclerosis

Over the past century, significant advances have been made in the development of animal models for atherosclerosis. Ignatowski (1908) first reported that rabbits fed with a diet rich in animal proteins (meat, milk, eggs) developed thickening of the intima with formation of large clear cells in the aorta. However, other investigators believed that the causal factor for atherosclerosis were lipids, but not proteins of the animal tissues (Wesselkin, 1913). Therefore the “cholesterol theory” of atherosclerosis was introduced. “Cholesterol theory” was one of the first hypothesis and almost all research activities in this field still remains at the center of atherosclerotic vascular disease development. However, administering certain drugs like poloxamer-407 (pluronic RF-127) (Wout, 1992; Palmer, 1997) and triton WR-1339 (tyloxapol) (Kuroda et al., 1977) are also known to induce hyperlipidemia. Atheromatous vascular lesions reported to resemble those of human atherosclerosis have been produced experimentally in rabbits, guinea pigs, hamsters, pigs, chickens, and monkeys by feeding large amounts of animal fat and cholesterol. Dietary cholesterol is needed for induction of atherosclerosis in experimental animal models (Katz et al., 1952), because of a strong association between certain types of dyslipidemia including hypercholesterolemia, hypertriglyceridemia, and combined hyperlipidemia and development of atherosclerotic lesions. This indicates an inevitable role for “cholesterol feeding” in atherosclerosis research. The atherogenic role of cholesterol has been tested in an ever increasing number of laboratory animals including wild-types, naturally defective or genetically modified animal models of atherosclerosis. The results of almost all these animal studies demonstrate increased serum/plasma cholesterol level, which is, therefore, a reliable method for induction of atherosclerosis.

The combination of vegetable oil and cholesterol has been shown to be one of the most effective atherogenic diets to induce atherosclerosis in non-human primates (Vles et al., 1964; Wissler, 1968). Several studies have shown that dietary saturated fat and cholesterol supplementation causes atherosclerosis/hyperlipidemia in rabbits (Kritchevsky et al., 1982; Hatipoglu et al., 2004), chicks (Castillo et al., 1996),
hamsters (Mangianpane et al., 1999) and rats (Sohn et al., 2005a; Almofti et al., 2006; Vijayakumar and Nalini, 2006a). Rats are generally hypo-responsive to dietary cholesterol; thus, hyperlipidemia and atherogenesis may only be induced in rats by high cholesterol/high fat diet containing cholic acid along with an antithyroid drug, thiouracil (Joris et al., 1983). Antithyroid drug induces clinical hypothyroidism with consequent decreased LDL-receptor activity and hypercholesterolemia (Joris et al., 1983; Meyer et al., 1989). In addition, cholic acid increases cholesterol absorption and suppresses cholesterol 7α-hydroxlyase activity that results in decreased cholesterol excretion.

The studies performed in this thesis were designed to evaluate the efficacy of EGCG in preventing or retarding atherosclerosis in a rat (Rattus norvegicus) model. The following six-pronged approach was adopted:

I) Isolation and purification of EGCG from green tea (Camellia sinensis) and testing of its in vitro antioxidant activity;

II) Induction of atherosclerosis in a rat model by feeding an atherogenic diet;

III) Evaluation of the putative anti-atherosclerotic effect of EGCG by evaluation of various biochemical parameters in rats fed an atherogenic diet;

IV) Determination of whether EGCG enhances the antioxidant status and reduces markers of oxidative stress in atherosclerosis;

V) Documentation of the expression of CRP and other inflammatory markers in rats fed an atherogenic diet alone and rats fed an atherogenic diet and treated with EGCG;

VI) Determination of alterations in expression of specific atherogenic-related genes in untreated and EGCG-treated rats.