CHAPTER 2

LITERATURE REVIEW

2.1 Studied Plant - *Rhodomyrtus Tomentosa*

Rhodomyrtus tomentosa or Kemunting is a wild shrub that fruits throughout the year. As a tropical plant, it can resist heat and high humidity temperature of tropical climate and rough soil especially in salty and acidic soil. *R. tomentosa* plant height can reach 3 meters. The flowers of *R. tomentosa* are pinkish white and sometimes in red colour. The berries of *Rhodomyrtus tomentosa* are roundish, purplish black when ripe, sweet and edible. Fresh berries are delicious eaten fresh with sweet and tad tangy. Due to its sweet characteristic, *R. tomentosa* fruits are favourite for birds and wild small animals. In Thailand, *R. tomentosa* often used as traditional medicine by folk people (Ruqiang and Yongly, 2006). They are similar in taste to blueberries and raspberries. The berries can be made into pastries like pies and jams. In Malaysia *R. tomentosa* plants are widely found in east coast of Malaysia especially in Terengganu and Johor where the soil is fertile for this kind of plant. The abundance of this kind of plant especially in remote areas is highly appreciated by the native people or the orang asli as they use as daily consumption.

Table 2.1: Scientific Classification of *Rhodomyrtus tomentosa*

Scientific Classification	
Kingdom	Plantae
Order	Myrtales
Genus	Myrtaceae
Family	Rhodomyrtus
Species	Rhodomyrtus tomentosa

2.2 Description

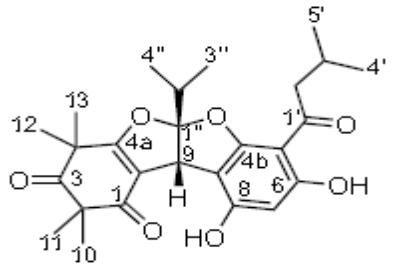
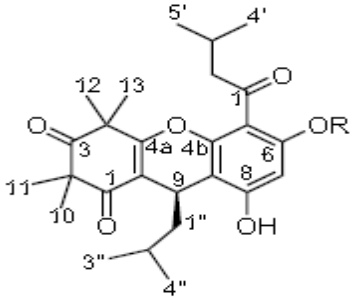
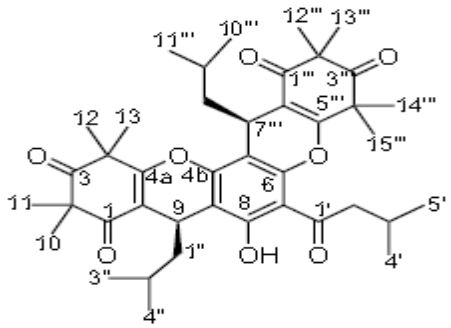
Figure 2.2.1: *Rhodomyrtus tomentosa* fruits

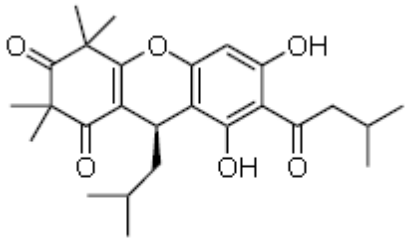
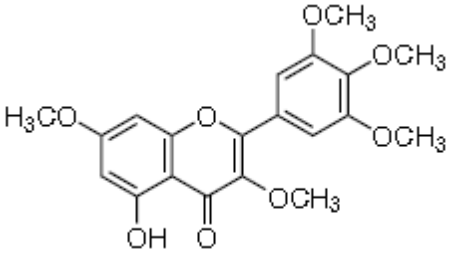
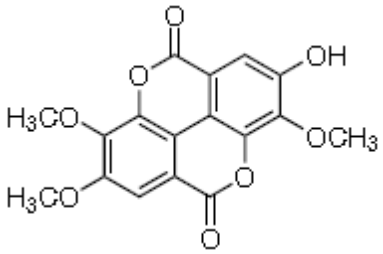
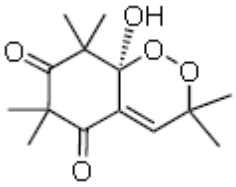
Figure 2.2.2: *Rhodomyrtus tomentosa* flowers

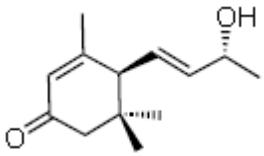
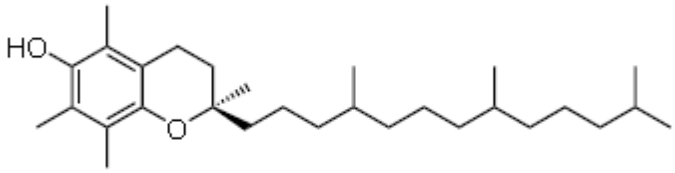
2.3 Chemical Constituent of *R. tomentosa*

According to Asadwahut and Wilawan 2008, acetone extraction of *Rhodomyrtus tomentosa* contains four new types of acylphlorogucinols named Rhodomyrtosones A, Rhodomyrtosones B, Rhodomyrtosones C and Rhodomyrtosones D (Asadhawut and Wilawan, 2008), Rhodomyrtone (Dachriyanus *et al*., 2002) together with bioactive compounds such as Combretol (Dachriyanus *et al*., 2004), 3,3',4-tri-O-methylellagic acid (Terashima *et al.*, 1990), Endoperoxide G3 (Crow *et al*., 1971), (6R,7E,9R)-9-hydroxy-4,7-megastigmadien-3-one (D'Abrosca *et al.*, 2004) and α-tocopherol (Crow *et al.*, 1971). Generally acylphlorogucinols in all four Rhodomyrtosones consists of the β-triketone moiety that is a rare chemical compound but commonly found in the plants of the Myrtaceae family. Others type of phytochemical compounds that have been identified including flavonoids (Dachriyanus *et al.*, 2004), tannins (Liu *et al.*, 1998) and triterpenes (Hui and Li, 1976). Table 2.3 shows list of chemical compounds of *R. tomentosa.*

Table 2.3: Chemical compounds isolated from *Rhodomyrtus tomentosa*

Compound	Chemical structure
Rhodomyrtosone A	 8,10-dihydroxy-5a-isopropyl-2,2,4,4-tetramethyl-7-(3-methylbutyryl)-5a,10b-dihydro-4H-benzo[b] benzo [4,5]furo[3,2-d]- furan-1,3-dione (Asadhawut and Wilawan, 2008)
Rhodomyrtosone B	 6,8-dihydroxy-9-isobutyl-2,2,4,4-tetramethyl-5-(3-methylbutyryl) -4,9-dihydroxanthene-1,3-dione (Asadhawut and Wilawan, 2008)
Rhodomyrtosone C	 7-hydroxy- 8,14-diisobutyl-2,2,4,4,10,10,12,12-octamethyl-6-(3-methylbutyryl) - 4,8,12,14-tetrahydro-5,13-dioxapentaphene - 1,3,9,11-tetraone (Asadhawut and Wilawan, 2008)

Compound	Chemical structure
Rhodomyrtone	 [6,8-dihydroxy-2,2,4,4-tetramethyl-7-(3-methyl-1-oxobutyl)-9-(2-methylpropyl)-4,9-dihydro-1H-xanthene-1,3(2H)-di-one] (Dachriyanus *et al*., 2002)
Combretol	 5-hydroxy-3,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chromen-4-one (Dachriyanus *et al*., 2004)
3,3',4-tri-O-methylellagic acid	 (Terashima *et al.*, 1990)
Endoperoxide G3	 (Crow *et al*., 1971)

Compound	Chemical structure
6R,7E,9R)-9-hydroxy-4,7-megastigmadien-3-one	 6R)-1,5,5-Trimethyl-6β-[(1E,3R)-3-hydroxy-1-butenyl]-1-cyclohexene-3-one;(6R,7E,9R)-9-Hydroxy-4,7-megastigmadien-3-one;(R)-4-[(R,E)-3-Hydroxy-1-butenyl]-3,5,5-trimethyl-2-cyclohexen-1-one (D'Abrosca *et al.*, 2004)
α-tocopherol	 (2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-(4,8,12-trimethyltridecyl)]-6-chromanol (Crow *et al.*, 1971)

2.4 Medicinal uses

The orang asli or the native people in Malaysia use the berries as remedy for dysentery and diarrhoea (Ong and Nordiana, 1999). Parts of roots and trunk are boiled in water for tonic in stomach ailment and as traditional medicine for post-partum (Verheij and Coronel, 1992). The local people of Indonesia have been using crushed leaves to treat wounds. Meanwhile the purplish colour of the berries due to high contents of tannin sometimes used to blacken teeth and eyebrows. In Vietnam, the fruits are used to produce local wine. Some have even documented the usage of *R. tomentosa* as remedy used to treat urinary infections (Wei, 2006).

Due to increasing resistance to antibiotics, new findings from plant's anti bacterial compounds have become a new alternative and hope to such resistance. In recent years, researchers have managed to extract a compound from *R. tomentosa* plant known as Rhodomyrtone, chemical structure [6,8-dihydroxy-2,2,4,4-tetramethyl-7-(3-methyl-1-oxobutyl)-9--methylpropyl)-4,9-dihydro-1H-xanthene-1,3(2H)-di-one] a type of acylphloroglucinol known as natural antibiotic for staphylococcal cutaneous infections (Jongkon *et al.*, 2008). This study will definitely put *R. tomentosa* plant extract as part of complimentary medicine studies especially in anti bacterial medicine. Other research on *Rhodomyrtus tomentosa* plant on Gram-positive bacteria such as *Bacillis cereus, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus Aureus, Staphylococcus Pyugene, Staphylococcus epidermidis, Streptococcus gordonii, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, and Streptococcus salivarius* have showed significant antibacterial activities (Voravuthikunchai *et al.*, 2007). Study by *Salni et al.*, 2002 has managed to purify Rhodomyrtone extract from *R. tomentosa* leaves using ethyl acetate and exhibited significant antibacterial effects, expressed as very low MIC (MIC= 0.19 1.56mg/ml) and MBC (MBC=0.39 25mg/ml) values against all Gram-positive bacteria. Generally,

much of the antibacterial activity can be attributed to Rhodomyrtone, a class of acylphloroglucinols consists of aromatic ring which has been reduced or has keto-enol form. The majority of these products are prenylated or farnesylated with acyl groups (Gibbons, 2004). Good activity against biofilm-forming and capsulated bacteria has been displayed by Rhodomyrtone, including *S. epidermidis* ATCC 35984 (biofilmpositive) and *S. pneumoniae* (capsule positive). Further study by Jonkon *et al.*, 2008 indicated the effectiveness of Rhodomyrtone isolated from *R. tomentosa* effectively attenuated *staphylococcus aureus* AT25923 with MIC value at 0.5µg/ml which is very close to vancomysin. This makes the use of Rhodomyrtone as an alternative agent for staphylococcal cutaneous infections. *R. tomentosa* plant also has a potential property of anti-hepatitis which inhibits the progress of hepatitis (Ruqiang and Yonglu, 2006). With this findings *R. tomentosa* has been hailed as alternative ways to reduce and prevent the problem of antibiotic resistance in bacteria. Besides that, haematological studies have shown that *R. tomentosa* extract has a profound effect on blood platelet aggression inhibitor and calcium antagonist (Mogata *et al.*, 1992). In pharmaceutical industry, *R. tomentosa* is part of formulations of skin-whitening, anti-aging and skin-beautifying agents (Miyake and Nojima, 2006).

2.5 Antioxidant

Antioxidant in the biological system play important role such as to prevent DNA and membrane lipids from oxidative damages and reduce the risk of several aging-associated health problems such as cancer and heart diseases. The major fat-soluble antioxidants are vitamin E and β-carotene (a vitamin A precursor). The major water-soluble antioxidant is vitamin C. These vitamins reduce LDL oxidation and preserve vasoreactivity by increasing endothelial nitric oxide release and reducing thrombogenicity (O'Keefe *et al.,* 1996). Antioxidant vitamins may also reduce the risk of plaque progression and rupture (Diaz *et al.,* 1997 and O'Keefe *et al.,* 1996).

Vitamin E

According to evidence from both epidemiological and clinical studies (Pham and Plakogiannis, 2005), tocopherols play an important role in reducing the risk of cardiovascular disease. In nature, there are four tocopherol isomers including α-, ß-, γ- and δ-tocopherols. Vitamin E can prevent the peroxidation of polyunsaturated fatty acid in membranes (Tacon, 1996). The most active and available form of vitamin E is α-tocopherol. Vitamin E is incorporated into lipoproteins and cell membranes, limiting LDL oxidation.Vitamin E is found in vegetable and seed oils, in wheat germ and, in smaller quantities, in meats, fish, fruits and vegetables. The recommended dietary allowance (RDA) of vitamin E is 30 IU per day (equivalent to 30 mg per day). It is difficult to obtain high doses of vitamin E in the average diet. Multivitamins usually contain 30 to 50 IU of vitamin E. Vitamin E was proven to inhibit oxidative towards Cu^{2+} mediated LDL (Andrikopoulus *et al.,* 2002). There was also a correlation between Vitamin E and increased plasma vitamin levels and enhanced in vitro oxidative resistance of LDL (Carpenter *et al.,* 2003).

Vitamin C

Dietary sources of vitamin C include citrus fruits, strawberries, cantaloupe, tomatoes, cabbage and leafy green vegetables. Cooking can destroy vitamin C; therefore, the vitamin is best obtained in raw foods or supplements. The RDA for vitamin C is 60 mg, but increased amounts are recommended for smokers, patients with healing wounds and pregnant or lactating patients. Vitamin C is able to scavenge free radicals and other reactive species that prevents oxidative modification of LDL. Intake of dehydrated juice concentrates has resulted in resistance of LDL to oxidation (Samman *et al.,* 2003). Similar positive results are seen when Vitamin C combined with Vitamin E and ß-carotene in patients with CVD (McKechnie *et al.,* 2002).

β - Carotene

Many carotenoids are known, but their functions are not yet understood. β - Carotene is a vitamin A precursor carried in plasma and LDL. Sources of dietary carotenoids include fruits, yellow-orange vegetables (e.g., carrots, squash and sweet potatoes) and deep-green vegetables (e.g., spinach and broccoli). No RDA has been established for carotenoids. It reduces oxidized LDL uptake but does not prevent LDL oxidation (McKechnie *et al.,* 2002).

Phenolic compounds

Phenolic compounds are found in plants. They can be categorized as simple phenolic, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. So far more than 4000 phenolic and polyphenolic compounds have been identified (Middleton and Kandaswami, 1994; Trease and Evans, 1989). Phenolic compounds play a vital role as antibacterial, anticarcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic,

and immune-stimulating agents (Larson, 1988). Several biological activities can be attributed to phenolic compounds such as strong antioxidant activity (Ho, 1992). The relationship of phenolic compounds and antioxidant activity has been reported in fruits, vegetables (Velioglu *et al.*, 1998).

2.6 Role of Antioxidants in prevention of atherosclerosis

In lowering down the oxidation of LDL, antioxidant plays a significant role by delaying or reducing the oxidation process (figure 2.6). Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation. The term "anti-oxidant" has been applied to a number of specific nutrients, most notably β-carotene, vitamin E, vitamin C and recently selenium (Garewal, 1997). Related to this, there is a growing interest in the profitability of plant-derived medicine studies as it carries antioxidant properties. Healing and preventing through the use of natural herbal remedies has long existed before the developmental and clinical investigation directed towards the cholesterol lowering potential occurred by plants. A number of chronic diseases including atherosclerosis involve oxidative damage to cellular components. Minimizing cellular oxidative damage is primary objective in preventive and treatment of atherosclerosis.

Antioxidant such as Vitamin C is an important compound as it can protect cells from oxidative damage therefore antioxidant from dietary consumption can prevent LDL oxidation and further give better protection against atherosclerosis (Esterbauer *et al*., 1992). Antioxidants may have direct interaction with reactive oxygen species (ROS) such as hydroxyl radicals or singlet oxygen molecules. Antioxidants may also form a chelating complex with transition metal such as iron (Fe^{2+}) and copper (Cu^{2+}) (Retsky *et al*., 1993). Besides that a more recent study has proved that antioxidants can alter total plasma and LDL level through changes in mRNA levels of 3-hydroxy-3-methylglutaryl-coA reductase (HMG-coA-R) and cholesterol 7α-hydroxylase (CYP7A1) (Rui *et al*., 2010).

Phenolic compounds have similar ability as antioxidants. Wide range of phenolic compounds found in plant such as simple phenols, phenylpropanoids, flavonoids, anthocyanins. Recently phenolic compounds gained more attention because

of their antioxidative, anti-inflammatory, antimutagenic and anticarcinogenic properties (Ho *et al.,* 1992). In the past, several phenolic compounds such as caffeic, ferulic, cinnamic, *p*-coumaric acids have been evaluated for their potential inhibitory effects on lipid peroxidation induced by peroxide anion (Toda *et al*., 1991). Further and in depth study of these phenolic acids were able to significantly reduce the peroxide formation in ghee and brain tumor. This was supported by an earlier observation that caffeic acid was about 1000-fold more effective than ferrulic acid in suppressing lipid peroxidation of rat brain homogenates in vitro (Graf, 1992).

Recent studies have found that actions of phenolic compound can either modulate the expression of COX-2 by suppressing the nuclear factor AP-1 (O'Leary *et al.,* 2004), regulate VEGF expression in aortic endothelial cells (Kenny *et al.,* 2004). This makes phenolic compound suitable in combating gene and factor that contribute to atherogenesis.

Vitamin is a good source of antioxidants. Vitamin E has been reputed as antioxidant for LDL lipid by increase in LDL oxidative resistance when exposed to strong oxidising conditions. For example Vitamin E in Cu^{2+} mediated oxidation, the oxidative effect of Vitamin E showed profound result (Andrikopoulus *et al.,* 2005). People who smoke tend to susceptible to atherosclerosis, a study of long term intake of Vitamin E in hypercholesterolemic smokers exhibited increase levels of autoantibodies against oxidised LDL (Heitzer *et al.*, 1993). One of the gene expressions in the initiation of atherogenesis is CD36 scavenger receptor expression. Deletion of this gene in successfully prevents the initiation of atherogenesis (Febbraio *et al.,* 2000). Inhibition of this gene has been linked to Vitamin E (Ricciarelli *et al.,* 2000) where it inhibits CD36 gene expression and macrophages (Devaraj *et al.,* 2001) in oxidised LDL in cells. Meanwhile a better prognosis showed in chronic smokers when a combination of high doses of Vitamin C and E, improving endothelial function, decreased plasma levels

plasminogen activator inhibitor-1 (PAI-1) and blood clotting factor such as von Williebrand factor (Antoniades *et al.,* 2003).

Some flavonoids in fruits contain anti-atherogenic properties. Resveratrol a type of flavonoid rich in grape has anti-atherogenic properties, owing to its inhibitory effect towards metalloproteinase-9 mRNA expression (Li *et al.,* 2003). Not only that, flavonoid such as resveratrol was reported to inhibit VCAM-1 mRNA expression which play an important factor in monocyte adhesion especially in the early development of fatty streak (Carluccio *et al.,* 2003).

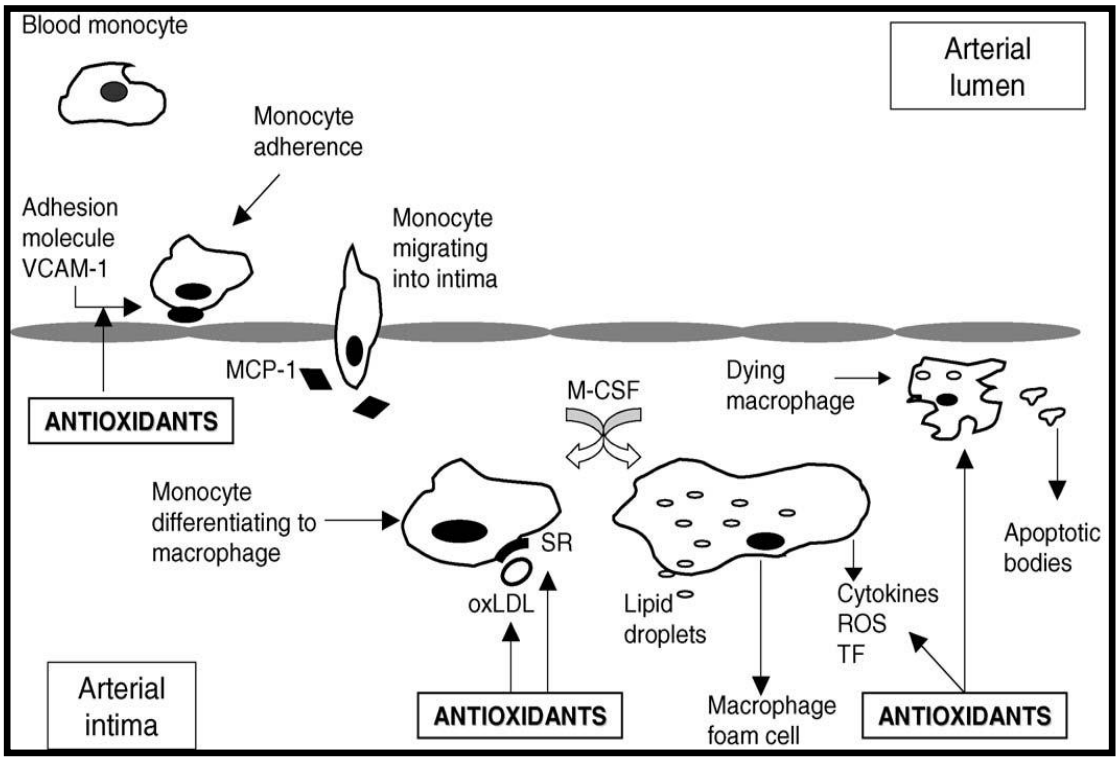


Figure 2.6: Role of antioxidants in atherogenic process (Kaliora *et al.,* 2006)

2.7 Lipid Peroxidation

Lipid peroxidation in biological system involves oxidative chain reaction in cellular levels (e.g lipid peroxidation in cell membrane). The chain is free mediated with many chemical mechanisms. Among biological molecules, lipids are the most susceptible to oxidative damage or peroxidation. Oxidative causes the deterioration of polyunsaturated fatty acids (PUFA) which widely found in abundance in cell membranes normally and starts a self-perpetuating chain reaction that produce a wide range of cytotoxic products such as malondialdehyde (MDA), 4-hydroxynonenal, and other by products. Lipid peroxidation is quantified by MDA content or by using a thiobarbituric acid reacting substances (TBARS) test (Ledwozyw *et al.,* 1986). This oxidative deterioration of lipids process involves the breaking of double bond structure of polyunsaturated fatty acids (PUFA) and propagation of lipid radical resulting in lipid damage. Lipid peroxidation causes many complications by altering the physiological function of cells.

Modification of LDL is related to lipid peroxidation. Elevation of LDL level may increase the risk of free radical oxidation, resulting in the generation of modified LDL that resulted in an increased uptake by macrophages (Retsky *et al.,* 1999). Modified LDL or oxidized LDL is termed as LDL exposed to an oxidant like free radicals or LDL that may have lipid peroxide or product of lipid peroxidation associated with it. LDL undergoes rapid physiochemical changes during oxidation such as electrophoretic mobility, change in buoyant density and chemical changes like loss of antioxidant, loss of PUFA and generation of lipid peroxidation products. Some of these changes have been correlated with altered biological properties**.** The initial concept of LDL oxidation is dependent on transition metals ions was postulated by Retsky *et al.,* 1999 when it was done and studied in cell media. Transition metal such as copper(II) has been the most commonly used inducer of oxidation for in vitro studies of LDL

oxidation (Frenkel and Meyer, 2000). LDL modification required traces metal such as iron or copper in the medium, generated dicarbonyl compounds that chemically react with thiobarbituric acid, and was inhibited by lipid-soluble antioxidants. By understanding LDL modification, we can hypothesised that lipid peroxidation plays a key role in rendering LDL atherogenic. This proposal was supported by chemical studies of oxidized LDL (Steinbrecher *et al.,* 1989) and by the immunohistochemical detection of protein-bound adducts of malondialdehyde and other end-stage lipid oxidation products in atherosclerotic lesions (Rosenfeld *et al.,* 1990).

The origin of the oxidative damage caused by lipid peroxidation is within the unique chemical nature of oxygen. When molecular oxygen is formed by the joining of two oxygen atoms, the outer valence shell electrons do not spin-pair, but remain as two unpaired electrons (Davies, 1996). Therefore, molecular oxygen is considered to be a true bi-radical. This radical character enables the oxygen to undergo unique oxidation and reduction chemistry. Aerobic organisms have been using this form of processes to gain essential energy by oxidizing carbon- and hydrogen-rich molecules using oxygen (Gutteridge, 1995). During reduction process, oxygen is univalently reduced, highly reactive intermediates is generated. Some of the known intermediates include the superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-) (Nakazawa, 1996). These agents have been shown to be responsible for oxidative damage by initiating lipid peroxidation.

As mentioned earlier, LDL is the main cholesterol transporter in the blood. In this form, cholesterol will be transported throughout human bodily cells and tissues because the higher the proportion of cholesterol, the lower the density of lipoprotein. Because LDL transports cholesterol to the arteries, increase levels are associated to atherosclerosis and thus myocardial infarction and stroke as mentioned. This elevation makes the LDL easily attack by natural occurring free radical in body

into oxidized form (De Groot, 1987). Research found that lipids containing polyunsaturated fatty acids are prone to be oxidized by oxygen derived free radicals. Gutteridge (1989) showed that free radicals play an important role in the development of tissue damage and pathological events. The conformational changes of lipoprotein (oxidized LDL) will lead to deposition in blood vessel (mainly aorta) as form cells as it can infiltrate easily into intimal layer of aorta and can cause complication as the elevation is not controlled.

The heart and cardiovascular tissues are more susceptible to lesion because the abundance of iron (Ferum) that present in cardiac muscle especially in cardiomyocytes (Gadja *et al.,* 2008). Iron also high in media and intima of smooth muscle. The amount of iron is about seven times higher in atherosclerotic lesion than in arterial wall (Thong *et al.,* 1996). Several factors have known to contribute the increase abundance of iron in cardiovascular organs. When erythrocytes were phagocytosed, the haemoglobin then will release iron in the form of ferrous ion (Fe^{2+}). The ferrous ion (Fe2+) somehow deposited in the atherosclerotic lesion area (Lee *et al*., 1999). Free ferrous ions Fe^{2+} together with hydrogen peroxide able to generate highly reactive hydroxyl radicals (Fenton's reaction). The oxidative state of iron is a good indicator of how much free radical is produced. The relationship of oxidative state of iron also has been studied in prostate cancer tissues (Kwiatek *et al*., 2005). Unlike iron, copper (Cu^{2+}) is able to generate hydroxyl radicals without the presence of hydrogen peroxide (Lynch and Frei, 1995). Besides lipid hydroxyl, radicals also attack apolipoproteins (Wagner and Heinecke, 1997). LDL oxidation was also reported when (Cu^{2+}) binds with ceruloplasmin (Ehrenwald *et al.*, 1994).

Removal of radical species with significant oxidizing character such the hydroxyl radical (OH^{-}) from from a polyunsaturated fatty acid (PUFA) signals the initiation of lipid peroxidation. This initiation can be stimulated with presence of ions or

metals, like iron, which increase the toxicity of H_2O_2 by promoting the formation of the hydroxyl radical (Hall, 1997). The removal of the allelic hydrogen from the PUFA forms a lipid radical (L). An immediate rearrangement occurs, forming a more stable lipid radical, whose dienes are conjugated (Gutteridge, 1995). In an aerobic environment this radical reacts with oxygen, giving rise to a lipid peroxyl radical (LOO). Propagation reactions can continue this process at this point by the lipid peroxyl radical abstracting an allylic hydrogen atom from another adjacent PUFA, resulting in a lipid hydroperoxide (LOOH) and a second lipid radical (L). This second lipid radical goes through the same reactions as the first reaction, producing additional lipid hydroperoxides. The propagation step continously goes through several eight rounds of peroxide generation on average, before a termination event occurs. The termination event can be the result of any reaction with another radical, protein, or compound that acts as a free radical trap, forming a stable end product (Davies, 1996).

The oxidation of LDL is inhibited by dietary antioxidant, i.e., ascorbic acid, alpha-tocopherol and carotenoids (Diplock, 1991). Two of the major antioxidant compounds that have received considerable attention are vitamin E (α-tocopherol) and vitamin C (ascorbic acid) and other forms of antioxidants. Cholesterol has been explored with greater advantage than any other form of compound to detect any oxidation process in cell membranes. This is different with unsaturated fatty acids, where cholesterol exists as a single molecular species, its oxidation products are regarded as much less complicated to isolate and characterize (Smith, 1981).

2.8 Role of lipid peroxidation

Oxidation of low-density lipoproteins (LDL) in the vessel wall is thought to be one of the steps involved in atherogenesis (Berliner and Heinecke, 1996; Steinberg *et al.,* 1989). Many lines of evidence suggest that LDL (figure 2.8), the major carrier of blood cholesterol, becomes atherogenic as a result of oxidation (Witztum and Steinberg, 1991 and Heinecke, 1998). It also is possible that oxidation of proteins, proteoglycans, or nucleic acids plays a role in atherosclerosis. Certain protein oxidation products are present at markedly higher levels in human atherosclerotic lesions than in normal aortic tissue (Heinecke, 1997).

Lipid laden foam cells is an important step in the pathogenesis of atherosclerosis because the oxidized LDL which cannot be recognized by liver LDL receptor and are taken up by macrophages cells leading to lipid-laden foam cells formation (Retsky *et al.,* 1999). Because oxidized LDL cholesterol is believed to have different properties than non-oxidized LDL cholesterol, LDL cholesterol accumulates in the cells that line the blood vessels. Various chemotactic and proliferative mechanisms lead to fatty streaks and later to atherosclerotic lesions. It is unknown whether oxidation of LDL cholesterol is important in both the initiation and progression of plaque or increases the risk for plaque rupture (Steinberg *et al.,* 1989).

The transformation of a clinically silent lesion into an atheromatous plaque proceeds by the migration of a variety of cell types, including transformed smooth muscle cells (Janero, 1991). Early in vitro experiments revealed that endothelial cells, smooth muscle cells, monocytes, and macrophages are the major cellular components of atherosclerotic lesions (Steinbrecher *et al.,* 1989). These cells extend the atheroma by the further oxidation of circulating LDL, the accumulation of lipid and the elaboration of collagen and elastin (Janero, 1991).

The development of an atherosclerotic plaque is thought to be initiated by injury to the vascular endothelium (Nicki *et al.,* 1991). In response to this injury, monocytes migrate into the subendothelial intima, where they differentiate into tissue macrophages. Lipid peroxidation and modification of LDL (Steinberg *et al.,* 1989) may be important early events in the transformation of these tissue macrophages into foam cells. The peroxidation of fatty acids yields specific aldehydic breakdown products that react with lysine residues in LDL and apolipoprotein (Steinbrecher *et al.*, 1989).

Oxidized LDL is chemotactic or monocytes and functions in their recruitment into the arterial intima, where they differentiate into tissue macrophages. Furthermore, oxidized LDL inhibits migration of macrophages away from the arterial wall. Because macrophages can oxidize LDL, the process can be self-perpetuating (Canfield *et al.,* 1992).

The uptake of LDL by macrophages occurs through three parallel mechanisms:

1. Unmodified or slightly modified LDL binds to the specific LDL receptor on the macrophage cell surface and is endocytosed. The activity of the macrophage LDL receptor is regulated by intracellular cholesterol content, and it is generally believed that this mechanism prevents foam-cell formation.

2. Oxidized LDL is internalized by the macrophage scavenger receptor, which has a broad specificity and is not regulated by cellular cholesterol content (Steinbrecher *et al.*, 1989).

3. Aggregated LDL and large LDL-proteoglycan complexes are directly ingested by phagocytosis. This is a very nonspecific pathway and, again, is not regulated by cellular cholesterol (Kovanen, 1991).

Endocytosed cholesterol can be returned to the circulation by esterification and donation to high-density lipoproteins (HDL) (Koren *et al*, 1991). The efficient operation of this reverse cholesterol transport blocks atheroma formation, and clinical disease is not manifested. Endocytosed polymeric LDL and LDL-proteoglycan complexes are metabolized slowly within macrophages and cholesterol from these sources is not efficiently removed by HDL transport.

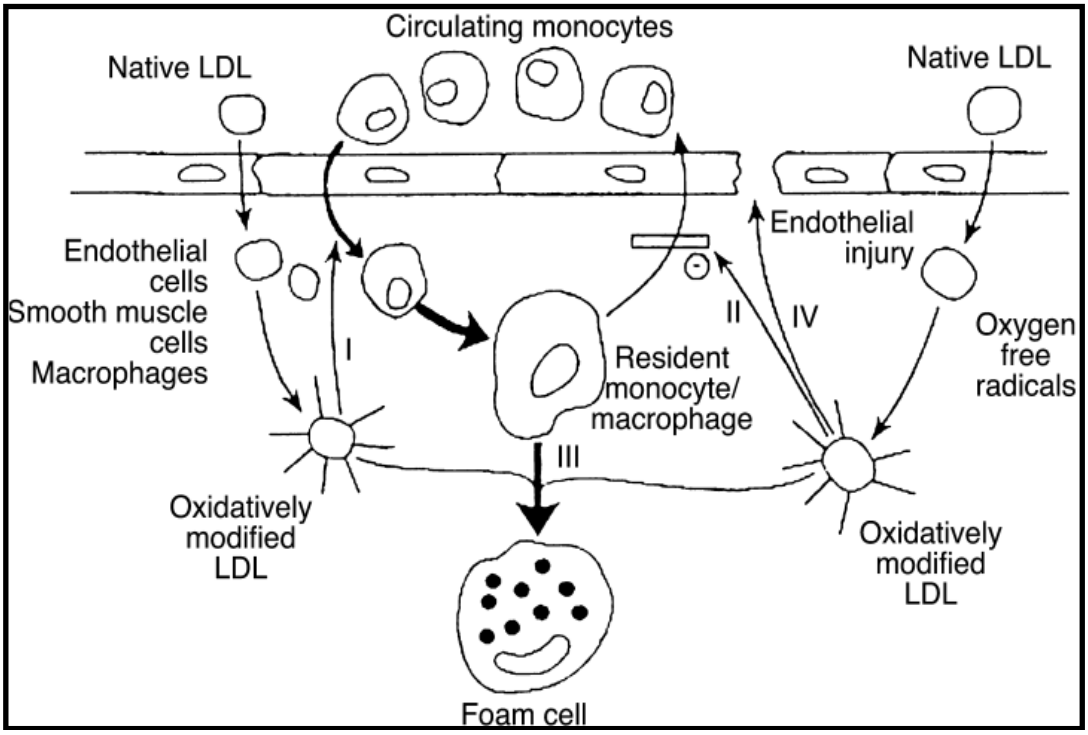


Figure 2.8: Role of oxidatively-modified low-density lipoprotein (LDL) in atherogenesis (Quinn *et al.,* 1985).

2.9 Cholesterol

Cholesterol is a waxy fat-like lipid compound found in every cell in human body. Cholesterol also presents in fatty foods that derived from animal origin. It is a member of a large group of substance called steroid (Hall, 1985). Cholesterol is normally found in the body in cell walls and membrane, vitamin D, hormones and fat digesting enzymes. This waxy substance has numerous functions. It is a major component of all cell membranes. It is required for synthesis of sex hormones, bile acids and vitamin D. it is also a precursor of steroid hormones produced by the adrenal cortex and gonads. Cholesterol can be in the form fluid state or in the form of gel. The different kinds of form help to balance, maintain and preserve the rigidity of cells (Vist and Davis, 1990). About 15% of blood cholesterol comes from diet and 85% made from acetyl-CoA in the liver circulating as lipoproteins.

The liver produces total daily cholesterol production in the body. Cholesterol originates from hepatic synthesis, diet, cell destruction and intestinal secretions. Generally, there are two different sources of cholesterol in human body. There are from our daily-consumed diet and from the synthesized internally by the liver termed as exogenous and endogenous cholesterol respectively. The endogenous cholesterol is made in the liver from the acetyl-CoA and to a lesser exert by other body cells, particularly the intestinal cells (Krause *et al.,* 1993). Some processed cholesterol is excreted via intestine which is eventually excreted in the faeces. Ultimately, cholesterol is recycled by the liver and reused in the formation of bile and bile salts (Theresa *et al.,* 1997). The hydrophobic characteristics of cholesterol require it to be transported in the form of particles made up to thousands of cholesterol molecules and other lipid bound to a protein (Campbell, 1999). This form of transportation is known as lipoprotein transport that has a hydrophobic core consisting of non-polar lipids coated with surfactants and proteins that are referred as apolipoproteins. This lipid carrier

protein, lipoprotein, transporting cholesterol via five major classes; chylomicrons, very-low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Two important lipoproteins that carry cholesterol are low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Their function is to distributing cholesterol throughout human organ mainly blood vessel and eliminating it from our body. Today, Cholesterol has been proven as contributor to a condition called atherogenesis (Steinberg, 2004). The relationships of highly saturated fatty acids, fat rich diet and low intake of dietary fibre and lack of physical exercise are considered of contributing factor in increasing cholesterol levels. By having to certain the level of serum cholesterol in physiological system, one able to predict the risk of having atherosclerosis as a well documented research has shown that total serum cholesterol levels at baseline can predict long-term cardiovascular mortality (Gupta *et al*., 2000).

2.10 Plasma Lipids

Lipid is one of a group of naturally occurring compounds that are soluble in solvents such as chloroform or alcohol but insoluble in water. The group includes fats steroids, phospholipids and glycolipids (Oxford Medical Dictionary, 2002). Lipid can be found throughout the living world, microorganism and in higher animal (Gurr and Harwood, 1991).

The major roles of lipids can be described as structural, storage and metabolic, although individual lipids may have several different roles at different times or even at one end the same time (Gurr and Harwood, 1991). They are important dietary constituent due to certain vitamins and essential fatty acids associate with them besides their high-energy value. Lipids also perform an important physiological task as the precursor of the hormone-like compounds and involves in the functioning of inflammatory reactions and the immune system's defence mechanism.

Lipids in human plasma mainly found as lipoproteins, triglycerides, phospholipids and cholesterol esters. All are in esterifies form with long chain fatty acids, but some fatty acids and cholesterol may present in plasma in free or non-esterified form. Free fatty acids are mobilized from adipose tissue by hormone-sensitive lipases and are transported in plasma bound to albumin to undergo utilization in the liver, skeletal and cardiac muscle or storage as triglyceride in adipose tissue.

2.11 Lipoprotein Transport in the Blood

Lipoprotein consists of a central core of hydrophobic lipid (triglycerides or cholesteryl esters) surrounded in a more hydrophilic coat of polar substances – phospholipids, free cholesterol, and associated proteins (termed as apolipoprotein). There are four main classes of lipoproteins differing in the relative proportion of the core lipids and in the type of apoprotein. They also differ in size and density. Each lipoprotein has a specific role in lipid transport in the circulation and there are different pathways for exogenous and endogenous lipids.

In the exogenous pathway, cholesterol and triglycerides derived from the gastrointestinal tract are transported in the lymph and then in the plasma as chylomicrons (diameter 100nm - 1000nm) to muscle and adipose tissue. These triacylglycerols are bundled into VLDL and further released into the circulation for delivery to the various tissues and organs (primarily muscle and adipose tissue) for storage or production of energy through oxidation. Here, on the vascular endothelial cells, the core triglycerides are hydrolised by a surface bound lipoprotein lipase (which requires one of the apoproteins as a co-factor) and the free fatty acids are taken up by the tissues. The chylomicrons remnants (diameter 30-50nm) still containing their full complement of cholesteryl esters, pass to the liver, bind to apolipoprotein receptors on hepatocytes and undergo endocytosis. Cholesterol is liberated within the liver cell and may be stored or oxidised to bile acids or secreted in the bile unaltered. Alternatively, it may enter the endogenous pathway of lipid transport VLDL.

In the endogenous pathway, cholesterol and newly synthesized triglycerides are transported as VLDL (diameter 30nm-80nm) to muscle and adipose tissue where the triglycerides are hydrolized and the fatty acids enter the tissues as described in figure 2.11. During the process, the lipoprotein particles become smaller (diameter 20nm-30nm), but still have a full complement of cholesteryl esters and

ultimately become LDL which provides the source of cholesterol for synthesis of steroids, plasma membranes and bile acids. Cells requiring cholesterol for the purpose of synthesise receptors and recognise LDL apolipoprotein which enable them to take up LDL by receptor-mediated endocytosis. Some drugs (e.g HMG-CoA reductase inhibitor) reduce the LDL concentration in the blood by stimulating the synthesis of these receptors in hepatocytes. LDL is the primary plasma carriers of cholesterol for delivery to all tissues. In contras of LDL, HDL will carry the excessive cholesterol back to liver for excretion. Cholesterol can return to plasma from the tissues in HDL particles (diameter 7nm - 20nm). Cholestrol is esterified with long chain fatty acids in HDL particles and the resulting cholesteryl esters are subsequently transferred to VLDL or LDL particles by a transfer protein present in plasma.

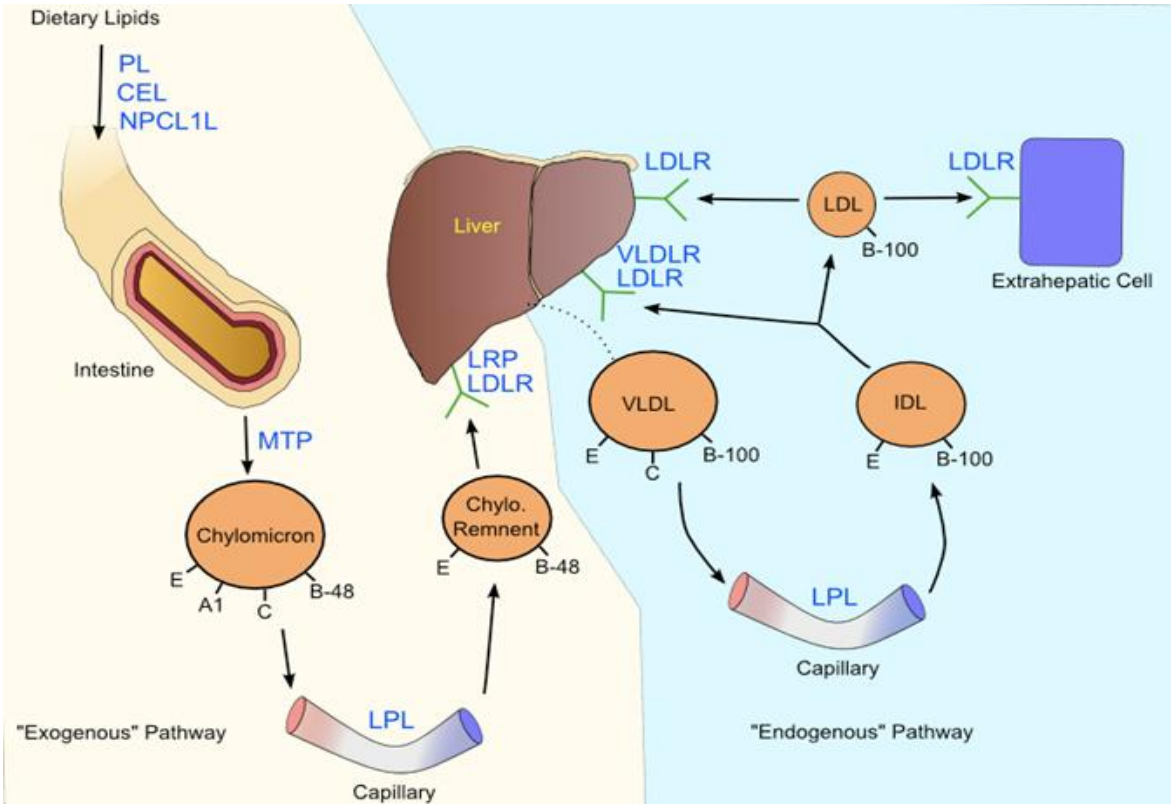


Figure 2.11: Schematic diagram of cholesterol transport (Tyler *et al.*, 2009)

2.12 Cholesterol Homeostasis in Physiological System

There are many diseases such as hyperlipidemia, atherosclerosis that has been associated with the disruption of cholesterol synthesis. High level of cholesterol is described as hypercholesterolemia. Therefore, determination of cholesterol in the body is essential for clinical diagnosis because elevated cholesterol level is a risk factor for atherosclerosis, myxoedema, nephrosis, and diabetes mellitus. Having high serum total cholesterol levels do correlate positively with increased risk of CVD (Stamler *et al.*, 2000; Zhang *et al.*, 2003).

Cholesterol homeostasis is maintained through combination of cholesterol synthesis, degradation and secretion. In order for the body to function as normal the amount of lipid output and lipid input must be maintained as it could. There are two major input pathways for cholesterol. The first one is receptor-mediated endocytosis of cholesterol carrying lipoprotein and the other one is *de novo* biosynthesis of cholesterol from acetate. LDL cholesterol is taken up by LDL receptor (LDLR) in a process called via receptor-mediated endocytosis which occurs in the liver. Meanwhile scavenger receptor known as (SR)B1 binds with high density lipoprotein (HDL) cholesterol. Cholesterol is then stored as cholesterol esters by activation of acyl-coA-cholesterol acyl transferase (ACAT). This process turns cholesterol into esterified cholesterol. Besides esterified cholesterol, cholesterol can also be changed into into very low density lipoprotein (VLDL) and further secreted into the blood. *De novo* biosynthesis of cholesterol involves numerous enzymatic reactions which occurred in the liver. Of which these numerous enzymatic reactions, the 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA-R) is the most important enzyme in cholesterol biosynthesis. The synthesis of cholesterol begins with one molecule of acetyl CoA and one molecule of acetoacetyl-CoA which then enters dehydration process to form 3-hydroxy-3methylglutaryl CoA (HMG-CoA) to form mevalonate by

the enzyme of HMG-CoA reductase. Mevalonate then converted to 3-isopentenyl pyrophosphates. 3-isopentenyl pyrophosphates undergo a series of steps with the end product is cholesterol.

Output processes is where hepatocytes disposes excess cholesterol into the blood circulation. This process involves two different pathways. Excess cholesterol is secreted directly into the canaliculus and by biosynthesis of bile acids. Almost 50% of cholesterol in the body is secreted by this pathway (Hyelemon *et al.,* 2001). In the liver, the biosynthesis of bile acid from free cholesterol occurs through two major synthetic pathways; the classic or neutral pathway and the alternative or acidic pathway.

The classic pathway is dependent on the presence of microsomal cholesterol 7α-hydroxylase (CYP7A1). The acidic pathway is regulated initiated by mitochondrial cholesterol 27-hydroxylase (CYP27A1). Under normal physiological condition, the classic pathway is a major pathway and the acidic pathway only represent minor to the overall bile acid synthesis (Chiang, 2004). Formation of hepatic cholesterol to bile acids is an important pathway so that excess cholesterol can be eliminated (Romero *et al.*, 2002).

In cells, there are complex mechanisms by which animal cells maintain the proper levels of intracellular lipid. When cellular cholesterol levels fall below the level needed, the cell with the help of enzymes will make necessary steps to increase cholesterol levels. This is achieved by making more of the mRNA transcripts that direct the synthesis of these enzymes.

Transcription process is regulated by Sterol Regulatory Element Binding Proteins (SREBPs) bind to the sterol regulatory element DNA sequence TCACNCCAC (Horton *et al.,* 2002; Eberle *et al.,* 2004). SREBPS located in the endoplasmic reticulum (ER) are synthesiezed as inactive precursors. Synthesized SREBPs then are released from the membrane of (ER) by two-step process and transported to the nucleus where

their main function as transcript factors by binding to sterol responsive elements (SRE) as shown in figure 2.12. The SREBP family consists of three subtypes: SREBP1a, SREBP1c and SREBP2, which control the expression of more than 30 genes required for the biosynthesis of cholesterol, fatty acids, triacylglycerides and phospholipids and also for cholesterol transport and glucose/insulin metabolism.

Different kinds of SREBPs have different kind of function. These different functions have been using studied in transgenic and knockout mice which proved that SREBPs have related but distinct roles; SREBP1 preferentially regulates fatty acid and glucose metabolism, while SREBP2 preferentially activates the LDLR gene and various genes required for cholesterol synthesis (Horton *et al.*, 2002; Eberle *et al.*, 2004)

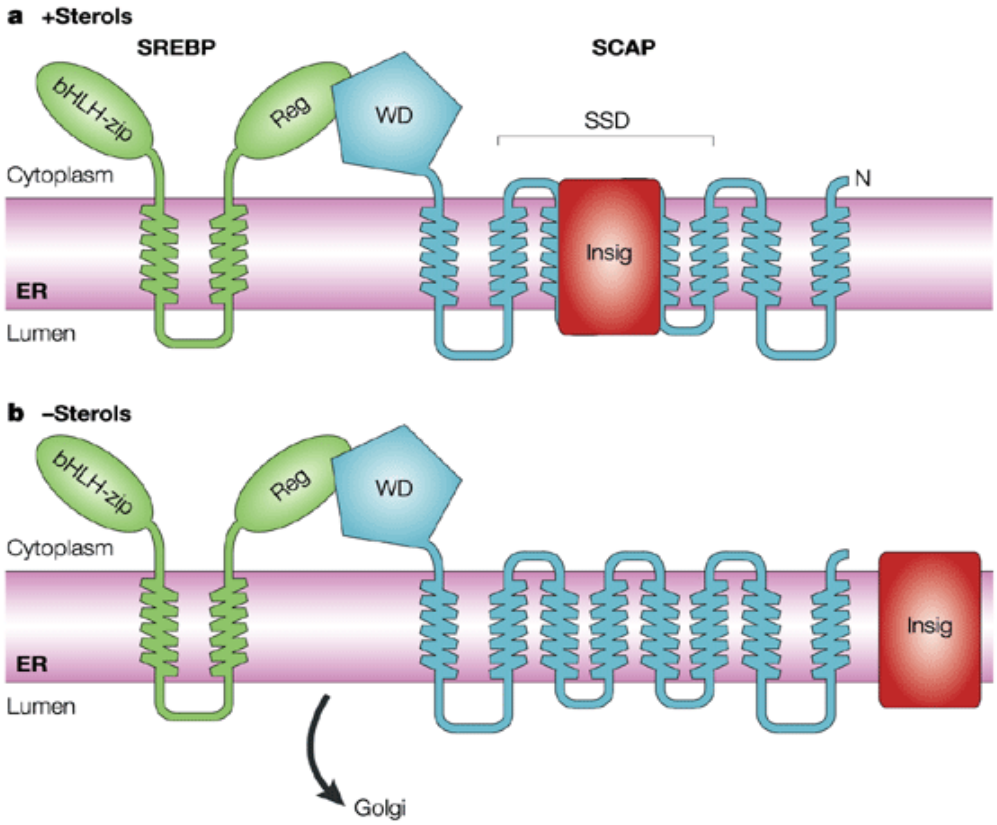


Figure 2.12: Transcription process by SREPs in endoplasmic reticulum (Robert, 2003)

2.13 Low Density Lipoprotein (LDL)

Low-density lipoprotein is the major cholesterol carrier in the blood (Gotto *et al.,* 1986). It carries newly synthesized cholesterol from the liver throughout the body. Normal value of LDL for human is 130mg/dL or 1.55mmol/L (NCEP, 1999). It is also the material that contributes most to the built up of plague in the arterial walls (Witztum and Steinberg, 1991).

As mentioned, too much LDL cholesterol circulates in the blood can slowly build up in the walls of the arteries feeding the heart and brain. Together with other substances it can form plaque, a thick, hard deposit that can clog those arteries. This condition is known as atherosclerosis. A clot (thrombus) is a condition that forms near this plaque which can block the blood flow to part of the heart muscle and cause a heart attack. A stroke results when a clot blocks the blood flow to part of the brain. A high level of LDL cholesterol (160 mg/dL and above) reflects an increased risk of heart disease. That's why LDL cholesterol is called "bad" cholesterol. Lower levels of LDL cholesterol reflect a lower risk of heart disease. Elevation of LDL is one of the risk factor in cardiovascular disease. Low density lipoproteins (LDL) are the main transporter of cholesterol in the human circulation and they play important part in cholesterol transfer and metabolism. LDL molecules have an average diameter of 22 nm, comprises about 170 triglycerides (TG) and 1600 cholesteryl ester (CE) molecules and the surface monolayer comprising about 700 phospholipid molecules and a single copy of apoB-100 (Esterbauer *et al*., 1992). LDL contains about 600 molecules of unesterifed cholesterol (UC), about one-third is located in the core and two-thirds in the surface (Lund-Katz and Phillips, 1986).

2.14 High Density Lipoprotein (HDL)

High-density lipoprotein is another group of lipoprotein circulating in human circulation system. HDL carries about one-third to one-fourth of blood cholesterol. The importance of HDL protection against atherosclerosis is shown in figure 2.14. The main function of this lipoprotein is to eliminate the excessive cholesterol level in circulating blood back to liver. In the liver, cholesterol will undergo metabolism process and lead to the elimination of the cholesterol from the body. Some experts believe HDL removes excess cholesterol from plaques and thus slow their growth.

Therefore, HDL cholesterol is known as "good" cholesterol because a high HDL level seems to protect against heart attack. A low HDL level (less than 40mg/dL) indicates a greater risk to atherogenesis. A low HDL cholesterol level also may raise stroke risk. It is advisable to have high level of HDL in blood. Based on the National Cholesterol Education Program, the normal level of HDL in human is 60mg/dL (3.36mmol/L).

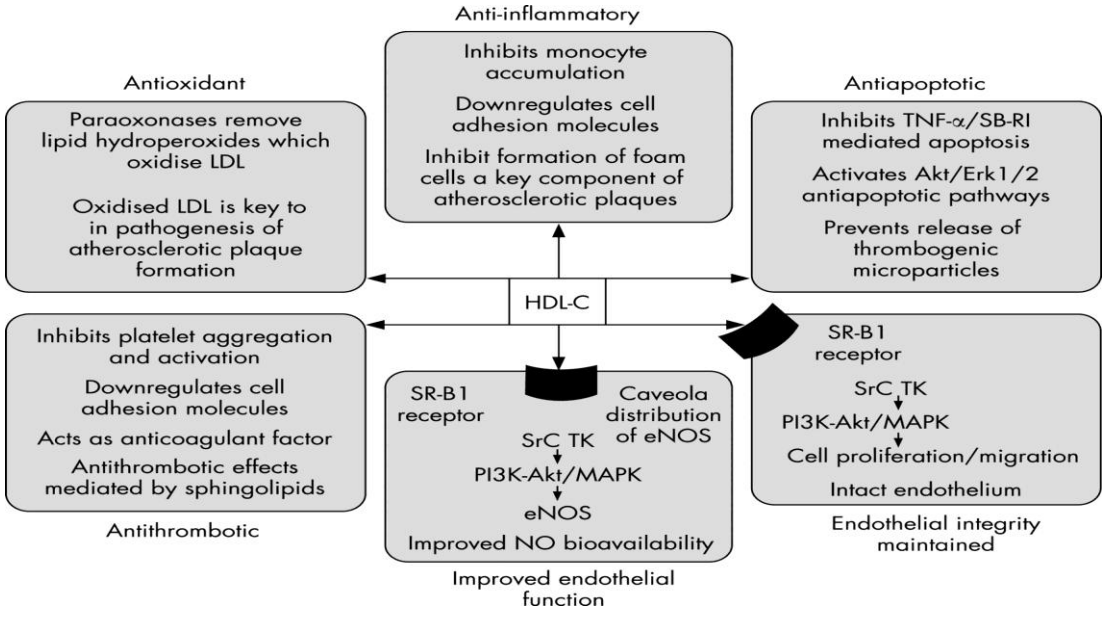


Figure 2.14: HDLs protection against atherosclerosis by several mechanisms (Hausenloy and Yellon, 2008)

It was recognized that plasma HDL correlates inversely with the incidence of atheroslcerosis (Miller *et al*., 1997; Gordon *et al.,* 1989; Shah and Amin, 1992; Wilson *et al*., 1989). Apolipoprotein AI (apoA-I) is the main protein component of HDL which plays a key role in the biogenesis and functions of HDL. Each HDL molecules has a density ranging from 1.063 to 1.21 g/ml. Generally HDL contains 45-55% apoproteins, 26-32% phospholipids, 15-20% esterified cholesterol, 3-5% free cholesterol, and approximately 5% triglycerides. HDL molecules have atheroprotective function that is unloading excessive cholesterol from peripheral tissues and its transport to the liver for catabolism, a process which is also known as reverse cholesterol transport (RCT) (Bruce *et al.,* 1989; Sviridov and Nestel, 2002). Reverse cholesterol transport is a multi series where excess cholesterol is removed from peripheral tissues, example removal of cholesterol from macrophage foam cells in the artery wall and returned to the liver for excretion from bile organ. Having a low level of HDL is an indicative of how prone the subject susceptible to atherosclerosis. High HDL level is said to be atheroprotective. Studies in human model showed that an increase in plasma HDL levels correlated with slower progression of atherosclerotic lesions (Gordon *et al.,* 1989; Gordon and Rifkind, 1989).

2.15 Triglycerides (TG)

The fats and oils that occur in plants and animals consist of largely of mixture of triglycerides or sometimes called triacylglycerols. These non-polar, water insoluble substances are fatty acid trimester or glycerol. Triglycerides are synthesis from the products of digestion of dietary fat. They are form which fat is stored in the body (Oxford Medical Dictionary, 2002).

Triglyceride is one of the most important classes of lipids. Almost 10% of human low density lipoproteins comprise triacylglyceride (Ritter *et al*., 1997). The major function of triglyceride is to supply and store the energy needed for metabolic functions and are therefore their most abundant class of lipids even though they are not component of cellular membrane. Excessive amount of fat in blood will be stored in adipose tissue and will be broken down into their component and used as energy source. This breakdown is aide by substances such as lipase, cortisol and epinephrine (Theresa *et al.,* 1997). Triacylglycerides determination is utilized in the diagnosis and treatment of many disorders in human. Elevated plasma triacylglyceride (TG) is regarded as a factor for developing cardiovascular disease (Egger *et al.,* 1999; Assmann, 2001; Le and Walter, 2007; Grundy, 1998). Triglycerides somehow able to interact with HDL cholesterol so that HDL levels fall as triglycerides level rise (Hokanson and Austin, 1996). This condition is not associated with elevated LDL cholesterol. The harmful imbalance of high triglycerides with low HDL level is also associated with obesity, insulin resistance and diabetes (Charles *et al.,* 1996). As low HDL cholesterol level is known to be harmful to the heart, so, this situation will lead to cardiovascular disease (Roger and Brian, 2002).

2.16 Hypercholesterolemia

As mention previously, cholesterol is required by human body in small amount to maintain cell integrity, manufacture hormones and others. When the level of cholesterol circulating in blood elevated then its normal value, it is termed as hypercholesterolemia. This condition can easily occur by the intake of diet high in saturated fatty acids as it strongly affecting the elevation of serum cholesterol.

The problem with cholesterol is that it can accumulate along the blood vessel and form "plaques" when it undergoes peroxidation process. Unhealthy cholesterol, particularly low-density lipoprotein (LDL), forms a fatty substance called plaque, which builds up on the intimal layer of arterial walls. Smaller plaques are soft, but older, larger plaques eventually tend to develop fibrous caps with calcium deposits. The long-term result is atherosclerosis. As the plaques get deposited in the blood vessel, mainly aorta, it can block or blood clot may form on top of the plaques and this can cause many problems to our body. This build up is called "atherosclerosis" or "hardening of the arteries".

Hypercholesterolemia is a silent disease. There are no symptoms until the resulting atherosclerosis causes complication. It narrows the arteries and can slow down or block blood flow to the heart. With less blood, the heart gets less oxygen. In this situation, there maybe chest pain, heart attack or even death. This process is aggravated which highly accelerated and enhanced by other risk factors, including high blood pressure, smoking, obesity, diabetes, and a sedentary life style.

The effects of cholesterol on the heart may involve more than just one target tissues such as the arteries. There is some evidence that high levels of cholesterol may affect the cardiac muscles and increase the risk for heart failure. Some drugs such as aspirin that provide protection for heart disease may be inhibited by the high cholesterol level. Mortality rates associated with coronary artery disease have dropped by over one-

half during the past 30 years. Medical experts have estimated that about 30% of the decline is due to better cholesterol management and medical therapy. Generally, the prevalence rate of mortality of people with high cholesterol levels is only 40%, however, and experts cannot yet define which people are most at risk from high cholesterol levels.

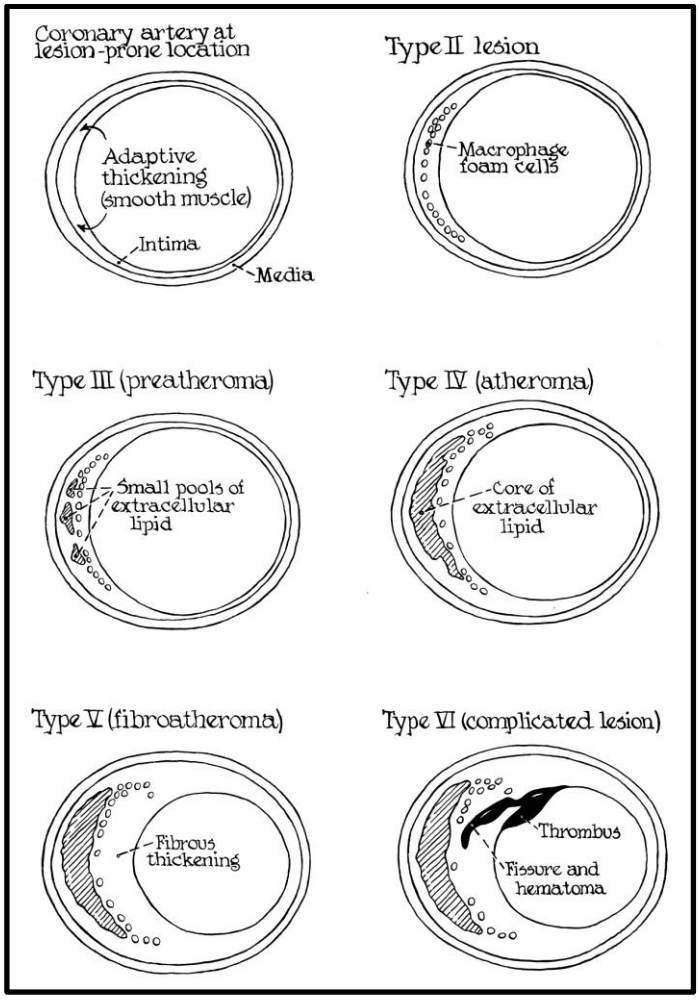


Figure 2.16.1: Type of aortic lesion **(**Stary *et al.*,1995)

The lesion that developed in the aorta can be divided into six types which are Type I (lesion), Type II (fatty streak), Type III (preatheroma), Type IV (atheroma), Type V (fibroatheroma) and Type VI (complicated lesion) as shown in figure 2.16.1 and figure 2.16.2. Type I lesions in atherosclerosis has been recognized as the very initial

changes and are represented as an increase in the infiltration number of intimal macrophages and the appearance of macrophages filled with lipid droplets (foam cells). Type II lesions manifested by the formation of fatty streaks lesion, the first grossly visible lesion, development of layers of macrophages foam cells and lipid droplets within intimal smooth muscle cells and minimal coarse-grained particles and heterogeneous droplets of extracellular lipid. Type III (intermediate) lesions are intermediate between Type II and advanced lesions. Types III lesions are characterised by plaque with small lipid cores but no visible calcification. Type IV lesions contain lipid core filled with macrophages, and foamy macrophages. Early fibrous cap developments can be seen at this stage with slightly little collagen. Collagen and elastic fibres form lipid core that surrounds lesions with fibrous later or cap in type V advanced lesions. In Type VI, most of the walls are indicated by aneurysm formation by severing the elastic lamina. In severe cases the plaques are indicated with thrombosis and haemorrhages (Stary *et al.*, 1995).

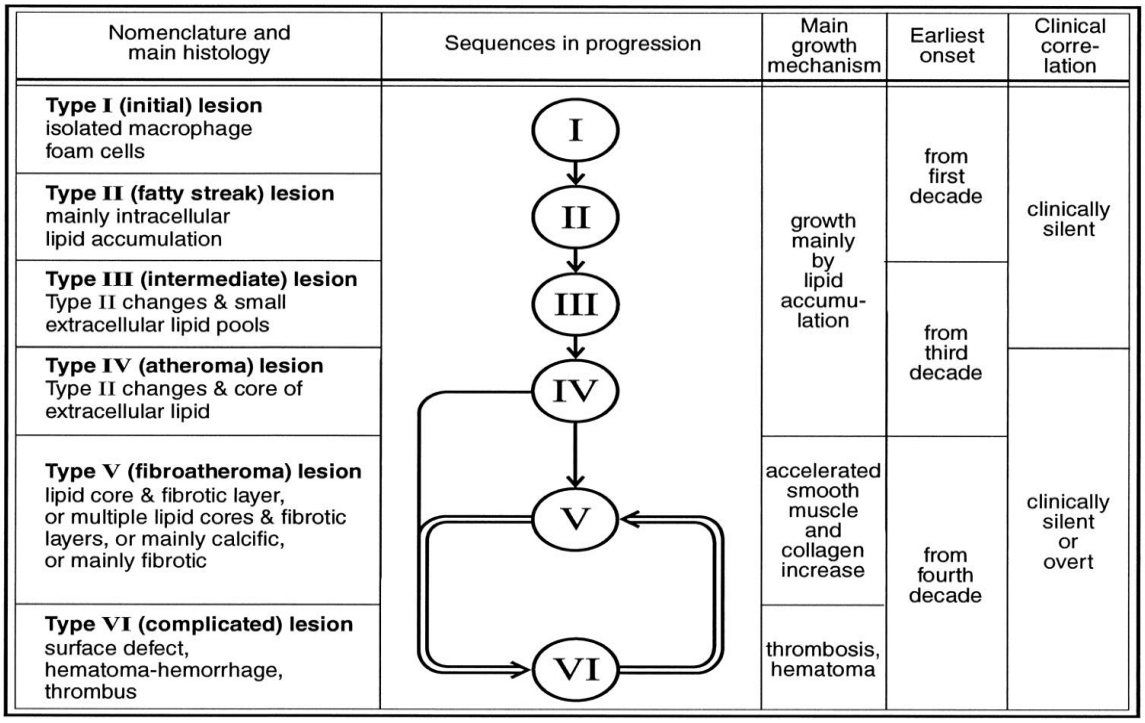


Figure 2.16.2: Pathways in evolution and progression of human atherosclerotic lesions **(**Stary *et al.*,1995)

2.17 Lipid Lowering Drugs as Treatment of Hypercholesterolemia

Several drugs are used to treat hypercholesterolemia in order to decrease blood LDL cholesterol (table 2.17). Drug therapy to lower lipids is only one approach to treatment and is used in addition to dietary management and correction of other modifiable cardiovascular risk factor.

Table 2.17: Frederickson/WHO Classification of hyperlipoproteinaemia Chol = cholesterol; TG = Triacylglycerides; LDL = low density lipoprotein; VLDL = very low density lipoprotein; ßVLDL = a quatitatively abnormal form of VLDL identified by its pattern on electrophoresis; + = increased concentration; NE = not related

Type	Lipoprotein Elevated	Chol	TG	Atherosclerosis Risk	Drug Treatment
I	Chylomicrons	+	+ + +	NE	None
II	LDL	+ +	NE	High	HMG-CoA reductase Inhibitor
IIb	LDL + VLDL	+ +	+ +	High	Fibrates, Nicotinic Acid, HMG-CoA reductase Inhibitor
II	ßVLDL	+ +	+ +	Moderate	Fibrates
IV	VLDL	+	+ +	Moderate	Fibrates ± (Fish Oil)
V	Chylomicrons VLDL	+	+ +	NE	None

A) HMG-CoA reductase inhibitors

The rate-limiting enzyme in cholesterol is HMG-CoA reductase which catalyzes the conversion of HMG-CoA to mevalonic acid (MVA). A class of drug that widely used as HMG-CoA reductase inhibitors is statins. There are six type of statins currently used in medical treatment; atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin and simvastatin. Of all type of statins, simvastatins is the most potent statins which the effects include lowering LDL and TG levels and

minor increase in HDL (Dani *et al.,* 2006). Simvastatin (figure 2.17) is an inactive lactone pro-drug which metabolised in the liver to its active form (Lucie *et al.,* 2008). The resulting decrease in hepatic cholesterol synthesis leads to increased synthesis of LDL receptors and thus increased clearance of LDL. In preliminary clinical trials in type IIa hyperlipidemia, statins have been shown to lower blood cholesterol by 33%, and when combined with colestipol, by 46%. The marked reduction in LDL-cholesterol could greatly reduce the risk of coronary artery disease, perhaps by as much as 50%-60% (Grundy *et al.,* 1988) when statins are given orally. They are well absorbed and extracted by the liver, their site of action and are subject to extensive presystemic metabolism. The benefit of statins not only in the treatment of lowering cholesterol, but as an antioxidant, anti-inflammatory and immunomodulatory has been reported (Werner *et al.,* 2002).

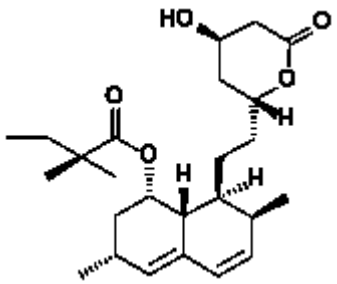


Figure 2.17: Structure of Simvastatin (HMG-CoA reductase inhibitors)

HMG-CoA reductase inhibitors or statins are well tolerated, mild and infrequent unwanted effects include gastrointestinal disturbance, insomnia and rash. More serious effects are rare but include severe myositis “rhabdomyolysis”, hepatitis and angio-edema (Khan *et al.,* 2007). Liver function tests should be monitored and patients warned to stop the drug and report for determination of plasma creatinine kinase activity if they develop muscle aches. In contrast to their usefulness in patients with heterozygous familial hypercholesterolaemia, HMG-CoA reductase inhibitors are

completely ineffective to patients with homozygous form of this disease who cannot make LDL receptors.

B) Nicotinic acid

Nicotinic acid is a vitamin which has been used in gram quantities as a lipid-lowering agent. Acipimox is a derivative of nicotinic acid which is used in lower dose and may have less marked adverse effect. These drugs inhibit hepatic triglyceride production and VLDL secretion which leads indirectly to a reduction in LDL. HDL is increased moderately. Other actions that could be advantageous in decreasing the risk of thrombosis are in increase in tissue plasminogen activator and thus increase thrombolysis and a decrease in plasma fibrinogen. Nicotinic acid is used in types II and IV hyperlipoproteinaemia. Long term administration is associated with reduced mortality (Canner *et al*., 1986). Clinical use is limited with some unwanted effects such as flushing, palpitation and gastrointestinal disturbances. High doses can cause disorders of liver function, impair glucose tolerance and increase the risk of gout.

C) Fish Oil

Omega-3 marine triacylglycerides reduce blood triglycerides concentration. A purified preparation of fish oil has been used in patients with severe hypertriglyceridaemia (e.g. types IV and V). The mechanism of fish oil on blood triglycerides is unknown. Fish oil is rich in highly saturated fatty acids including eicosapentaenoic and docasahexaenoic acids and has other potentially important effects including inhibition of platlet function, prolongation of bleeding time, anti-inflammatory effects and reduction of plasma fibrinogen (Lorenzo *et al.,* 1983). Eicosapentaenoic acid substitutes for arachidonic acid in cell membranes and gives

rise to 3-series prostaglandins and thromboxanes and 5-series of leukotrienes (Bell *et al.,* 1998). This probably accounts for their effects on haemostasis since thromboxane A_3 is much less active as a platelet aggregating agent than thromboxane A_2, whereas prostaglandin I_3 is similar in potency as an inhibitor of platlet function to prostaglandin I_2 (prostacyclin) (Michinao *et al.,* 1995).

D) Fibrates

Several fibric acid derivatives "fibrates" are used clinically including bezafibrate, gemfibrozil, fenofibrate and clofibrate (Backes *et al.,* 2007). These drugs have a marked effect in lowering VLDL and hence triglyceride with a modest approximately 10% increase in HDL. They are therefore used in patients with raised triglycerides as well as raised cholesterol (e.g type IIb and type III). The mechanisms of fibrates are incompletely understood but they stimulate lipoprotein lipase, hence increasing hydrolysis of triglycerides in chylomicron (Chapman, 2003). They also probably reduce hepatic VLDL production and increase hepatic VLDL uptake (Chapman, 2003). Fibrates also reduce plasma fibrinogen (Schonfeld, 1994) and improve glucose tolerance (Elkeles *et al.,* 1998).

2.18 Malondialdehyde (MDA)

The first step in lipid oxidation is the abstraction of a hydrogen atom from a fatty acid and subsequent oxygen involvement gives a peroxy radical. Peroxide then eventually decomposes to lower molecular compounds, mainly malondialdehyde (Ledwozyw *et al*, 1986). Most of chain cleavage products developed from monohydroperoxides are molecules belonging mainly in two groups, such as simple hydrocarbons and short chain aldehydes. These unsaturated aldehydes then will pass thorough a process called autoxidation to form volatile compounds. This process makes aldehydes undergo cleavage to form shorter chain and sometimes other chemical groups are added. In 1991 Mao *et al*., 1991 were one the first researchers to discover the antioxidative capacity of compounds to inhibit the Cu^{2+} -induced oxidation of LDL by measuring the levels of TBARS formed with and without the presence of antioxidants in the reaction suspension. Since then, many markers of lipid oxidation besides TBARS have been utilized in LDL oxidation experiments including conjugated dienes, total lipid peroxides, oxysterols, hydroxyl ad hydroperoxy fatty acids, aldehydes, lysophosphatides, oxygen consumption, head space hexanal, and disappearance of polyunsaturated fatty acids (Puhl *et al*., 1999; Frankel *et al.*, 1992).

Among all these substances, Malonaldehyde (MDA) has becoming the subject of interest in studies of lipid peroxidation despite its complex and uncleared origin. So far, MDA and conjugated dienes method have been most widely used in studies examining the antioxidant properties of compounds in the Cu^{2+} LDL system (Liyana and Shahidi, 2006). MDA may be formed also in some tissues by enzymatic processes with prostaglandin precursors as substates. Human platelet activation normally involved an enzyme known as thromboxane synthetase, MDA along with thromboxane A_2 was generated from prostaglandin endoperoxides (Hecker and Ullrich, 1989). MDA is able to form adducts with free amino acids and many more with

proteins. MDA introduces cross-links in proteins that may induce profound alteration in their biochemical properties. During oxidation process, malondialdehyde can be determined by TBA method. The TBARS test is a colorimetric technique in which the absorbance of a red chromogen formed between TBA and malondialdehyde is measured (Rhee, 1978).