Introduction
1. INTRODUCTION

Circulatory system, which plays a major role in maintaining homeostasis i.e. stable internal environment, consists of heart which acts as the life pump and the units of systemic circulation such as arteries, veins and capillaries. In average human, nearly 7572 litres of blood travel daily through about 96,000 kilometres of blood vessels. Aorta, the largest artery of the circulatory system is a root systemic artery that carry oxygenated blood pumped from the left ventricle of the heart via aortic valve (Maton, Anthea et al., 1995). This biological system that involves in pumping and channelling of blood is affected by a number of diseases, such as arrhythmias, atherosclerosis, hypertension, stroke and others. In 2010 almost 32% (788,000) of death was caused by cardio vascular disease (CVD), the annual death caused by CVD substantially increased from the early 20th century and remains high. Though, the death rate due to CVD has declined from 1999, patient affected by CVD have been increased in recent years (NHLBI, 2012).

Among the various CVDs, atherosclerosis is a chronic vascular inflammatory disease can be ranked as number one cause of mortality and morbidity in both developed and developing nations (WHO, 2011). Atherosclerosis is more vulnerable to occur in aorta, the main artery of the heart which is an elastic artery that contains three layers namely the innermost layer or tunica intimal layer made up of endothelial cell and a lining of internal elastic lamina. The tunica media is made up of smooth muscle cells and elastic fibres arrayed in rough spiral layers. The outermost coat or tunica adventitia is a tough layer consisting mainly of collagen fibres. Atherosclerosis is an insidious process that may persist for many years before clinical manifestations. Early atherosclerotic lesions are characterised by deposition of lipids, oxidation of lipids, appearance of macrophages and T
lymphocytes in the tunica intima. During initial stages, the LDL lipoproteins start to accumulate in tunica intima of arteries on the sites that are predisposed to develop atherosclerosis where it is oxidatively modified, which induces endothelial cells and express leukocyte adhesion molecules and chemokines that recruits monocytes (Boring et al., 1998). These oxidatively modified oxLDL is engulfed by macrophages and gets transformed into foam cells.

These Cluster of lipid laden cells become macroscopically visible as fatty streaks (Davies et al., 1993). Progressively, these fatty lesions transform to fibrolipid plaques, as intimal smooth muscle cells proliferate and deposit extracellular matrix, mainly collagen. In a subsequent stage, the advanced lesion has a characteristic microanatomy with a core of extracellular lipid separated from the tunica media by smooth muscle cells and covered at
the luminal side by a thick fibrous cap. In simple, atherosclerosis may be manifested in four major steps starting with initiation of endothelial activations and inflammations, followed by promotion of intimal lipoprotein deposition, retention, modification and foam cell formation, with progression of plaque growth, enlargement of necrotic core, fibrosis, thrombosis and remodelling, all of which leads to acute events like myocardial fibrosis, unstable angina and sudden coronary death (Hu et al., 2014). Therefore the key initial event in atherosclerosis is endothelial activation, which represents a switch from quiescent phenotype to one that involves host defense response. Indeed most of the cardiovascular risk factors that are associated with the disease such as smoking, hypertension, diabetes mellitus and hypercholesterolemia activates molecular machinery in endothelium which results in expression of chemokines, cytokines and adhesion molecules.

1.1. Risk Factors Of Atherosclerosis

Risk factors that can accelerate the process of atherosclerosis can be divided into two major categories as modifiable and non-modifiable risk factors.

1.1.1. Modifiable

Modifiable risk factors are generally associated with the life style of a person and which can be modified. These include

- Diabetes or impaired glucose tolerance (IGT)
- Tobacco smoking
- Vitamin B6 deficiency
- Dietary iodine deficiency and hypothyroidism, which cause elevated serum cholesterol levels.
- Diets rich in saturated and trans-fatty acid.
1.1.2. Non-modifiable

Non-modifiable risk factors include

- Advanced age
- Gender
- Family history of premature atherosclerosis
- Low socioeconomic status

1.2. Hypercholesterolemia

Among the risk factors mentioned in the previous section hypercholesterolemia plays the major role in development of atherosclerosis (Rerkasem et al., 2008). Hypercholesterolemia is clinically described as presence of high levels of LDL cholesterol in the blood. It is a form of "hyperlipidaemia" (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (elevated levels of lipoproteins in the blood). Even though cholesterol plays a vital role in cell metabolism and structure, excessive amount of cholesterol in cells can destroy membrane functions or result in atherosclerotic damage of blood vessels (Martini et al., 2007). Certain genetic causes of abnormal increase in cholesterol and triglycerides are known as hereditary hyperlipidemia (Ackermann et al., 2001). Environmental factors include obesity and dietary habits. In most cases elevated cholesterol levels are associated with continuous ingestion of high cholesterol (Bedogni et al., 2005), stress and smoking cigarettes.

1.3. Metabolism of Cholesterol

Cholesterol is a sterol, since cholesterol is insoluble in water; it is transported in the blood plasma within protein particles (lipoproteins). Lipoproteins are classified by their
density: Very low density (VLDL), Low density lipoprotein (LDL), Intermediate density lipoprotein (IDL) and high density lipoprotein (HDL). Cholesterol and cholesterol esters are relatively minor, but important components of VLDL (Davis and Vance, 1996). Rather than just being present in lipoproteins cholesterol is used for membrane biogenesis, cell growth, synthesis of steroid hormones and bile acids (Liscum and Munn, 1999). In plasma, the free cholesterol present in HDL is esterified by lecithin cholesterol acyltransferase (LCAT) and apoA-1 activates LCAT (Burgess et al., 2002), this converts cholesterol into cholesteryl ester. Those nascent preβ HDL particles eventually become mature spherical particles after accumulating sufficient cholesterol ester. Spherical HDL is then acted on by CETP, which facilitates the transport of CE to the storage lipoproteins, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), in exchange for TG (Tall, 1990).

Cholesterol is transported in the bloodstream in HDL and ApoB containing lipoproteins almost exclusively as cholesterol esters. More than half of the apo B associated cholesterol is internalized by hepatic LDL receptors, which are located in clathrin-coated pits on the hepatocyte cell surface (Meddings and Dietschy, 1986). In rats, 60–70% of HDL-C is removed by SR-BI-mediated selective uptake (Pittman and Steinberg, 1984). The human homolog of SR-BI, termed CLA-1, exhibits similar tissue distribution (Calvo et al., 1997), binding properties for a wide spectrum of plasma lipoproteins, and identical cholesterol transfer capacities as those of murine SR-BI (Calvo, et al., 1998). Data from a human hepatic cell model demonstrate that CLA-1 is responsible for the selective uptake of cholesterol from HDL and LDL particles (Rhainds et al., 2003). Furthermore, recent epidemiological studies have identified single-nucleotide
polymorphisms in the CLA-1 gene that are associated with abnormal plasma lipid levels and lipoprotein composition (Acton et al., 1999). These studies indicate that SR-BI/CLA-1 play a pivotal role in RCT and the metabolism of the cholesterol component of both HDL and apoB-lipoproteins in humans. Cholesterol is synthesised in almost all the cells, excess cholesterol from non-hepatic peripheral tissues is transported back to liver by reverse cholesterol transport. The first step in reverse cholesterol transport involves the transfer of cholesterol from cell membranes to acceptor lipoprotein particles in the extravascular fluids.

Two mechanisms control this transfer of cholesterol. In the aqueous diffusion model, cholesterol molecules spontaneously disassociate from cell membranes, traverse the intervening aqueous space by diffusion, and then incorporate into acceptor particles. This
process is driven by the gradient of free cholesterol between cells and the aqueous medium (Yokoyama. 2000). It is also transported by interaction between lipid poor HDL and cell surface ABCA1, which promotes cellular phospholipid and cholesterol efflux by loading free apolipoprotein A-I (apoA-I) with these lipids. Apo A-I binds to cell surface and ABCA1 transfers cholesterol in an energy dependent process on to apoA-I containing HDL (Oram and Vaughan. 2000).

The association between cholesterol and atherosclerosis is unequivocal, the link for this association is familial hypercholesterolemia, which is an autosomal dominant disorder that affects approximately 1 out of 500 persons in general population and these patients develop premature atherosclerosis at the age of 15 (Keaney, 2000). The lowering of serum cholesterol is increasingly recognised as essential in the prevention of coronary heart disease and other atherosclerotic disease (Durrington, 2003). In people with very high cholesterol (e.g. familial hypercholesterolemia), diet is often insufficient to achieve the desired lowering of LDL and lipid lowering medications which reduce cholesterol production or absorption are usually required. If necessary, other treatments such as LDL apheresis or even surgery (for particularly severe subtypes of familial hypercholesterolemia) are performed (Ito et al., 2012). LDL cholesterol remains the primary target of therapy for the prevention of coronary heart disease. Increasing research attention over the past decade has been devoted to the heterogeneity of LDL particles and the atherogenicity of lipids and lipoproteins other than LDL such as small dense LDL particles and oxidized LDL (Carmena et al., 2004).
1.4. Role of LDL Oxidation and Oxidative Stress in Atherosclerosis

Reactive Oxygen Species (ROS) are generated in aerobic organisms during physiological or pathophysiological oxidative metabolism in mitochondria. ROS may react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue, thereby interfering with cell function. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems. Impairment in the oxidant/antioxidant equilibrium provokes a situation of oxidative stress and generally results from high production of ROS (Wendel, 1987). Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases.

In a study conducted by Goldstein and his colleague in 1979 showed, that incubation of LDL with normal macrophages possessing normal LDL receptor does not support foam cell formation. In fact, high concentrations of LDL generally led to down regulation of LDL receptor as receptor mediated endocytosis is tightly regulated. In later studies, it was found that oxidative stress in vascular wall cells (endothelium, smooth muscle cell and macrophage) is responsible for oxidative modification of LDL and oxidatively modified LDL seems to be responsible for macrophage recruitment, retention and foam cell formation in atherosclerotic plaque (Henriksen et al., 1983; Henriksen et al., 1981). Oxidised LDL is shown to be cytotoxic to a variety of cells in cell culture and it does so by increasing cholesterol accumulation inside the cell especially in macrophages (Witzium and Steinberg, 1991).

According to current concept LDL oxidation is a complex processes in which lipids and proteins undergo oxidative changes and form complex products (Parthasarathy et al.,
The oxidative modification of LDL has been arbitrarily divided into three stages. The first is known as the initiation of lipid peroxidation and involves the initial formation of radical species within the particle. The second stage is known as propagation stage of oxidation and represents the portion of LDL oxidation involving a chain reaction, in which each radical produced gives rise to more than one subsequent radical.

The final stage of LDL oxidation is known as decomposition into reactive aldehydes and ketones. This leads to modification of apoB moiety of LDL and changes the net charge of LDL. LDL can be oxidised in vitro by copper ions and by various cultured cells like endothelial cells, smooth muscle cells and macrophages (Steinberg et al., 1989).
In *vitro*, LDL that is modified by copper ion or acetylation is readily engulfed by macrophage and smooth muscle cells (Sparrow *et al.*, 1989).

### 1.5. Role of Inflammation in Atherosclerosis

About 50% of patients develop atherosclerosis in the absence of systemic hypercholesterolemia. Recent literature states that atherosclerosis is not just about accumulation of lipid, despite the important role of cholesterol in atherosclerosis, many individuals who experience myocardial infarction have cholesterol concentrations at or below the National Cholesterol Education Program thresholds of 200 mg/dL for total cholesterol and 130 mg/dL for LDL cholesterol (Castelli, 1988 and Wong *et al.*, 2012). Putative antigens, heat shock proteins, components of plasma lipoproteins (Lp), and various microbial structures induce an inflammatory process that on its own may generate
the atherosclerotic plaque formation (Millonig et al., 2002). Atherosclerosis is a chronic inflammatory disorder and dyslipidaemia acts as an inducer.

Oxidised LDL is the major causative agent of inflammation in atherosclerotic plaque. For example minimally modified LDL gets retained in the sub-endothelial space. The key early inflammatory response to retained apoB containing Lipoproteins, not only enhances the oxidative modification of the lipoprotein but results in activation of overlying endothelial cells (Mestas and Ley, 2008) and smooth muscle cells to synthesize and secrete monocyte chemotactic protein-1 (MCP-1) (Cushing et al., 1990; Steinberg et al., 1989; Liao et al., 1997) that interact with cognate chemokine receptors on monocytes and promote directional migration. Macrophages are important effector cells of the immune system that are formed in response to an infection or accumulating damage. Macrophages are now recognized as key pathophysiologic agents in wide spread disease processes associated with chronic inflammation such as atherosclerosis (Moore and Tabas, 2011).
1.5.1. MCP-1

Monocyte chemoattractant protein-1 (MCP-1/CCL-2) is a member of C-C chemokine family that have two adjacent cysteine’s near the amino terminus which is a potent chemotactic factor for monocytes. CCL-2 is a monomeric polypeptide, with a molecular weight of approximately 13kDa. There are two distinctive regions of primary structure that are critical for CCL-2 biological activity. Mutation in both regions severely decrease CCL-2 activity (Deshmane et al., 2009). CCL-2 is produced by many cell types including endothelial, fibroblast, smooth muscle and monocytes (Cushing et al., 1990; Barna et al., 1994) and it regulates the migration and infiltration of monocytes. However monocyte/macrophages are found to be the major source of CCL-2 (Yoshimura et al., 1989) and it is critical for the initiation and development of atherosclerotic lesions. There studies evidently prove that inhibition of CCL-2 signaling in hypercholesterolemic mice have greater effect on reducing lesion size (Combadière et al., 2008) and its over expression is associated with greater progression of atherosclerosis by increasing both macrophage numbers and oxidized lipid accumulation (Cipollone et al., 2001).

1.5.2. Tumour Necrosis Factor-α (TNF-α)

Tumour Necrosis Factor-α is one of the pleotropic cytokine involved in systemic inflammation and it is produced by macrophage, vascular endothelial cells and smooth muscle cells. TNF-α is produced as 233 amino acid long type II transmembrane protein, which is later processed to soluble 17 kDa protein (Palladino et al., 2003). It exerts many of its effects by binding as a trimer, to 55kDa cell membrane receptor termed TNFR-1 (Idriss et al., 2007). In a study conducted on APOE*3-Leiden mice crossed with TNF-α deficient mice found that TNF-α modulates lesional cell death by increasing necrosis and
decreasing the incidence of apoptosis, moreover TNF-α progresses the lesion towards a more advanced phenotype (Boesten et al., 2005). TNF-α can induce the production of cytokines, chemokines and increases the expression of adhesion molecules on endothelial cells and leukocytes, leading to the recruitment of monocytes and infiltration into subendothelial space of arteries (Ross, 1999; Getz, 2005; Tedgui, 2006).

1.5.3. Interleukin-1β (IL-1β)

IL-1β is a member of Interleukin 1 family of cytokines; it is produced by activated macrophages as a pro-protein, later processed by caspase-1 to its active form. It is an important mediator of inflammatory response and is involved in cell proliferation, differentiation and apoptosis. Elevated levels of IL-1β result in secretion of chemokines and other cytokines (eg, IL-6), increased expression of adhesion molecules, activation of endothelial and smooth muscle cell proliferation, macrophage activation, and increased vascular permeability. This cascade promotes atherosclerosis and plaque destabilization (Mann, 2002). In addition IL-1β contributes to the development of tissue damage by stimulating cell proliferation and the release of matrix metalloproteases (Faten Merhi-Soussi et al., 2005).

1.5.4. Interleukin-6 (IL-6)

IL-6 is a both proinflammatory and anti-inflammatory cytokine that is produced by Macrophage, T-cells and smooth muscle cells in the tunica media of vascular tissue (Jurgen Scheller et al., 2011). IL-6 signals through type I cytokine receptor complex consisting IL-6Rα chain (CD-126) and the signal-transducing component gp130 (Heinrich et al., 1998). Interleukin-6 (IL-6) cytokines and their signalling events have been shown to
contribute to both atherosclerotic plaque development and plaque destabilisation via a variety of mechanisms. These involve the release of other pro-inflammatory cytokines, oxidation of lipoproteins by phospholipases, stimulation of acute phase protein (APP) secretion, the release of prothrombotic mediators, and the activation of matrix metalloproteases (MMPs) (Yudkin et al., 2000).

1.5.5. Transforming Growth Factor-β (TGF-β)

The TGF-β superfamily of cytokines contains more than 30 structurally related polypeptide growth factors, they all share three distinct domains: N-terminal signal domain which associates with precursor molecule for proper cellular secretory pathways; a propeptide domain, which supports folding or dimerization of the mature cytokine; and a long C-terminal TGF-beta-like domain which is highly conserved across the super family (Hinck et al., 1996). TGF-β cytokines play a major role in cardiovascular system development and angiogenesis (Bobik, 2006). In a study single allele deletion of TGF-β gene, which results in 50% decreased expression of TGF-β in vessel media lead to reduced Smooth Muscle Cell differentiation and increased susceptibility to endothelial cell activation and vascular lipid lesion formation in response to pro-atherogenic stimuli like lipid rich diet (Grainger et al., 2000).

1.6. Cell Adhesion Molecules

The vascular adhesion molecules have a major role in atherosclerosis, they are involved in recruiting immune cells into the walls of arteries. There are several steps in leukocyte recruitment into vascular tissues initially depends on selectin tethering and
rolling, which triggers adhesion via chemokines and their (Hidalgo et al., 2007; Zarbock et al., 2007).

1.6.1. VCAM-1

VCAM-1 is an immunoglobulin-like adhesion molecule expressed on activated endothelial cells (Osborn et al., 1989). VCAM-1 binds to α4β1 integrin, which is constitutively expressed on lymphocytes, monocytes, and eosinophils. Interestingly, VCAM-1 can mediate both rolling-type adhesion and firm adhesion, depending on the avidity status of α4β1 integrin (Chen et al., 1999). Although, it is structurally similar to ICAM-1 and other endothelial adhesion molecules, VCAM-1’s pattern of regulation is unique. VCAM-1 is not expressed under baseline conditions but is rapidly induced by pro-atherosclerotic conditions in rabbits, mice, and humans (Cybulsky and Gimbrone, 1991; Nakashima et al., 1998; O’Brien et al., 1993), including in early lesions. Initially, it was unclear whether VCAM-1 was simply a marker for atherogenesis or whether it acts in this disease pathway. Studies with cytokine-activated cultured endothelial cells and reconstitution assays with purified recombinant VCAM-1 protein (Chen et al., 1999) suggested that VCAM-1 could mediate robust adhesion of α4β1 expressing cells, even under shear flow.

1.6.2. ICAM-1

Another member of the immunoglobulin superfamily, ICAM-1, is also involved in atherosclerosis, presumably through the regulation of monocyte recruitment into atherosclerosis-prone areas. ICAM-1 expression is elevated in atherosclerosis-prone aortas and is regulated by proinflammatory stimuli (Kita et al., 2001). OxLDL induces
endothelial ICAM-1 expression, however, not only oxLDL but also native LDL increases the expression of ICAM-1 on HUVECs and elevates monocyte adhesion to the activated endothelium (Smalley et al., 1996). In vivo administration of native LDL into Ldlr−/− recipient mice model showed that native LDL is able to induce ICAM-1 as well as VCAM-1 expression. However in a double knockout mice (Icam1−/− and Apoe−/−) model, ICAM-1 absence had no significant protection against plaque formation and no lesion size difference was observed (Manka et al., 2001).

1.6.3. P-Selectin

P-selectin with a molecular weight of 140 kDa is the largest of the known selectins family of proteins. It contains nine consensus repeats (CR) and extends approximately 40 nm from the endothelial surface. Within minutes of stimulation of endothelial cells in vitro by inflammatory mediators, such as histamine, thrombin, or phorbol esters, or hypoxia, Weibel–Palade bodies are mobilized and degranulate their von Willebrand factor. The expression is short-lived, reaching its peak after only ten minutes. Additional synthesis of P-selectin is brought about within two hours by cytokines such as interleukin-1 (IL-1) or tumor necrosis factor α (TNF-α). The primary ligand for P-selectin is PSGL-1 (P-selectin glycoprotein ligand-1) which is constitutively found on all leukocytes. Other ligands for P-selectin include CD24 and uncharacterized ligands. The transient interactions between P-selectin and PSGL-1 allow leukocytes to roll along the venular endothelium. Accordingly, P-selectin is largely responsible for the rolling phase of the leukocyte adhesion cascade (Andrew et al., 2003).
1.6.4. **E-Selectin**

E-selectin, also known as CD62 antigen-like family member E, it is expressed only on endothelial cells activated by cytokines. E-selectin is also detected on human atherosclerosis-prone ECs and on the surface of fibrous and lipid-containing human plaques. In mice, genetic deficiency of E-selectin (encoded by the Selegene) leads to reduction in the lesion size (Collins *et al.*, 2000). E-selectin found on ECs is also stimulated by inflammatory cytokines such as tumour necrosis factor TNF-α and interleukin IL-1β (Stocker *et al.*, 2000).

1.7. **Apoptosis**

Apoptosis or programmed cell death is a key regulator of physiological growth control and regulation of tissue homeostasis. However apoptosis is not limited to cell elimination during development, it is also involved in many pathophysiological processes, the evidence for apoptotic cell death in primary atherosclerotic lesion was found by Isner *et al.* (1995). Although apoptosis can be triggered by several different stimuli, apoptotic signalling within the cell is transduced mainly via two defined molecular pathways: the death receptor pathway and the mitochondrial pathway (Canbay and Friedman 2004). The end point of both the intrinsic and extrinsic pathways is activation of a wide variety of intracellular proteases especially a group of proteolytic enzymes called caspases and endonucleases that ultimately degrade the cellular constituents (Tagashira *et al.*, 2000). Apoptosis can be divided into three non-distinct phases: an induction phase, an effector phase, and a degradation phase.

The induction phase depends on death- inducing signals to stimulate pro-apoptotic signal transduction cascades. Proteins of the Bcl-2 family play a fundamental role in
mammalian cell death. The Bcl-2 family is composed of over a dozen proteins that have been classified into three functional groups. The members of the first group are anti-apoptotic and have four short, Bcl-2 homology domains (BH1–BH4). They also have a C-terminal hydrophobic tail that allows them to localize to the outer surface of the mitochondria. This group includes Bcl-2 and Bcl-xL. The members of the second group are pro-apoptotic and similar in structure to the first group; however, they do not possess the BH4 domain. This group includes Bax and Bak. The members of the third group are a heterogeneous collection of proteins that share a BH3 domain; some are divergent homologues of Bcl-2 and Bax, such as Bid, whereas others are likely to possess a BH3 domain through convergent evolution (Hengartner and Bryaut, 2000).

Caspases cascade has been demonstrated to be involved in apoptotic signal transduction and execution (Huang and Strasser, 2000). Human caspases 1-10 have been described and activation of the caspases cascade is involved in chemical-induced apoptosis (Hinds et al., 2003). During apoptosis, protease such as caspase gets activated with an obligatory cysteine residue within the active site and cleave peptides adjacent to an aspartic acid residue (Antonsson et al., 2001).

Caspase 3 is situated at pivotal junction in apoptotic pathway that cleaves vital cellular protein or activates additional caspases by proteolytic cleavage. Caspases 3 usually exist as an inactive pro-caspase 3 that becomes proteolytically activated by multiple cleavages of 32 kDa precursor to generate the 20/11 or 17/11kDa active forms in cells undergoing apoptosis (Karbowski et al., 2002). Thus, appearance of 17 kDa proteins may consider as one of the active forms of caspase-3, which may then execute apoptosis (Nechushtan et al., 2001).
In atherosclerotic plaque endothelial cells, smooth muscle cells and macrophages all of them undergo apoptosis, but apoptosis of macrophage is evident in all stages of atherosclerotic plaque (Andrés et al., 2012). Macrophage apoptosis is thought to promote the formation of the necrotic core in advanced atheroma, thus increasing plaque vulnerability and the risk of thrombotic vascular disease (Schrijvers et al., 2007).

1.8. Natural Therapeutics

The drugs that are usually used to treat atherosclerosis include fibrates (which reduce cholesterol absorption from intestine) and statins (which inhibits cholesterol synthesis) and that too with lot of side effects (Hayward et al., 2010).

These synthetic medicines are good enough to provide symptomatic relief, but are associated with undesirable side effects and high costs. Therefore, it is more and more need for developing further effective and harmless alternative therapies to lower serum cholesterol. Population aging, modern life style changes and limitations/side effects of modern medicine have caused people to look for new methods to improve their health. Biologically active nutraceutical with medicinal properties to reduce cholesterol levels are being identified recently (Muto et al., 2001). Typical examples include plant sterols (Ostlund et al., 1999) fiber (Queenan et al., 2007), soy (Hori et al., 2001), and green tea catechins (Kuo et al., 2005). Recently, much attention has been focused on the protective biochemical function of naturally occurring phytochemicals and on the mechanisms of their action (Singh et al., 2008).

Among the various phytochemicals, green tea fascinates a grand interest because they have been and are still being taken on a daily basis by the young and old in Asian countries, where the life expectancy is high with low rates of mortality due to CVD. There
is considerable evidence that dietary antioxidants, particularly polyphenols can help to prevent the development of cardiovascular diseases (Yang and Landau, 2000). Phenolic compounds and flavonoids have pharmacological properties such as antioxidant, anti-mutagenic, anti-thrombotic, anti-inflammatory, anti-cancer and hypolipidemic (Son and Lewis, 2002; Monforte et al., 1995). Various plants contain a number of phenolic compounds, including phenolic acids (e.g., gallic acid and caffeic acid), flavonoids (e.g., quercetin, epicatechin, rutin, myricetin, luteolin, naringenin, and silybin), phenolic diterpenes, and volatile oil (Huang et al., 2010).

Green tea and its major constituent polyphenols are best known for their various biological and pharmacological properties including anti-oxidative (Song et al., 2005), antibacterial (Stapleton et al., 2004), antitumor (Mukhtar and Ahmad, 2000) and antiviral activity (Song et al., 2005). In several studies natural bioactive compounds isolated from plant sources give a lot of promise in treating various disease without any side effects (Yang et al., 2001). In green tea polyphenols, EGCG epigallocatechin 3-gallate constitutes major proportion (Khan et al., 2006). In recent years, the number of studies investigating the roles of EGCG has risen dramatically. EGCG has shown to have anti-hypercholesterolemic, anti-inflammatory, antioxidant and antibacterial properties (Ikeda et al., 1992; Dona et al., 2003; Osada et al., 2001; Zhang et al., 2004). EGCG is shown to offer definite cardiovascular health benefits (Sueoka et al., 2001). Studies have shown that green tea extracts (GTE) and its major component, epigallocatechin-3-gallate (EGCG) can inhibit the invasion of human umbilical vein endothelial cells (Yamakawa et al., 2004), prevent tumour blood vessel growth (Pfeffer et al., 2003) and offer protection against mutagenesis (Lee et al., 2003). The anti-diabetic application of GTE has been validated in
animal models with insulin resistance (Wu et al., 2004). GTE comprises other biological activities such as anti-inflammatory (Dona et al., 2003) and anti-Aging activity as well (Hsu et al., 2005). EGCG has also been found to be capable of strongly inhibiting the replication of HIV in cultured peripheral blood cells (Fassina et al., 2002).

1.9. Polyphenols

Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals, chocolate, dry legumes and beverages (Manach et al., 2004). These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens and may also contribute to the bitterness, astringency of the food. These denote a huge gamut of substances having aromatic ring(s) bearing one or more hydroxyl moieties, with over 8000 variants (Harborne, 1980). Researchers have explored that these molecules are very good antioxidants and may neutralize the destructive reactivity of undesired reactive oxygen/nitrogen species produced as by-product during metabolic processes in the body which are mediated by the presence of para-hydroxyl group (Harborne and Baxter, 1999). Epidemiological studies have revealed that polyphenols provide a significant protection against development of several chronic diseases such as cardiovascular diseases (CVDs), cancer, diabetes, infections, aging, asthma etc (Pandey and Rizvi, 2009).

Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans (Spencer et al., 2008). The flavonoids subclasses include: flavonols, flavones, flavanols,
isoflavones, anthocyanidins and others (Spencer et al., 2008). Though a large number of polyphenolic compounds are present in dietary sources, a few polyphenols are more well-known and studied extensively, namely resveratrol, curcumin and the catechins.

1.9.1. Green Tea Polyphenol - EGCG

The potent antioxidant actions of green tea are associated with EGC and EGCG because their chemical structures have galloyl moieties, which is absent in other catechins. Moreover, only EGCG has two triphenolic groups in its chemical structure which is responsible for its anti-oxidative potency (Osada et al., 2001).
Numerous in vitro studies reveal that EGCG scavenges a wide range of free radicals including singlet oxygen, superoxide anions, peroxyl radicals and also the most active hydroxyl radicals, which initiate lipid peroxidation (Nanjo et al., 1996).

1.9.2. Metabolism and Bioavailability of EGCG

Green tea consumption results in the plasma catechin concentration between 0.2% and 2% of the ingested amount, with a maximal concentration after 1.5 to 2.5 hours after consumption. The half-life of EGCG is about 5 hours (Pietta et al., 1998). EGCG cannot be recovered in urine, whereas EGC can be partly recovered. The efficient metabolism of the flavonoid EGCG has been studied in humans, using radioactively labelled catechins (Hollman et al., 1996). Bioavailability is defined as the rate and extent to which the active ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action (Yang et al., 1999). It is mainly dependent on the solubility, permeability as well as the stability in the GIT and biotransformation before reaching the blood through oral route. EGCG bioavailability is less than 2% of the oral dose administered in rats (Chen et al., 1997). EGCG is rapidly conjugated in plasma, however free EGCG was found in tissues (Lambert et al., 2003). EGCG is mainly excreted through the bile; however, urinary excretion also takes place when administered through oral route or parenteral route (Yang et al., 1998). In rats, zinc absorption inhibition has been observed, although results for copper remain unclear. Polyphenols also affect the bioavailability of sodium and aluminium; it does not interfere with that of calcium, manganese, or magnesium (Hollman et al., 1996).
1.10. Nano Particles in Drug Delivery

Nanotechnology is becoming the driving force behind a variety of evolutionary and revolutionary changes in the medical field. The impact of nanotechnology on drug delivery has helped to improve the efficacy of available therapeutics and will likely enable the creation of entirely new therapeutic entities. Nanomedicine will not only improve conventional therapies, but also bridge the shortcomings of conventional medicine to help people on both global and individual levels (Shi et al., 2010).

Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some microorganisms, yeast and fungi (Illum, 1998). The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose. These units combined by β - (1,4) glycosidic linkages, forming a long chain linear polymer. Although chitin is insoluble in most solvents, chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric, and citric acid (LeHoux and Grondin, 1993; Peniston and Johnson, 1980). It is insoluble in phosphoric and sulfuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation. Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation. Even though the cardiovascular benefits of EGCG has been proven, its rapid excretion from the body reduces its bioavailability therefore its usage as a cardio protective agent is questionable (Chen et al., 1997). The therapeutic effect of EGCG is limited by its poor systemic absorption following oral consumption (Lin et al., 2007; Huo et al., 2008). Most of the ingested EGCG does not get into blood, since absorption takes place in the small gut and substantial amount pass
through large intestine where it undergoes further degradation (Lee et al., 2002). EGCG stability is affected by many factors like temperature, metal ions Ca\(^{2+}\) and Mg\(^{2+}\) and in vivo EGCG is rapidly methylated by catechol-O-methyltranferase (Hong et al., 2010). Though EGCG has shown many promising protective effects clinically relevant levels of EGCG in plasma may not be reached in vivo through oral ingestion of EGCG (Shammas et al., 2006).

Studies indicate that almost only 5% of EGCG is absorbed into the systemic circulation of rats following oral administration (Chen et al., 1997; Zhu et al., 2000; Lin et al., 2007). In order to maximise the therapeutic utility of EGCG there is a need to enhance its oral absorption (Ghosh and Scheepen, 2009). Nano drug delivery may provide a way for this hurdle, in the present study EGCG is encapsulated into chitosan nanoparticle for effective absorption.