1. Introduction

The world Health Organization (WHO), reported that approximately one third of deaths, globally, were attributable to cardiovascular disease (CVD) (WHO 2015, Moss and Ramji, 2016). Cardiovascular disease ranks first in the global cause of death, computing for 17.3 million deaths per year, a number that is forecasted to grow to more than 23.6 million by 2030. CVD claims more lives than combining all forms of cancer. The source for this health statistics comes from the annual compilation of the government sources such as the American Heart Association, the Centers for Disease Control and Prevention, the National Institutes of Health and finally updated as Heart Disease and Stroke Statistics Update (Mozaffarian et al., 2014). For the first time since the British Heart Foundation was formed, cardiovascular disease is no longer the leading cause of death in the United Kingdom. In 2012, CVD caused 28% of all deaths while cancer caused 29% (Townsend et al., 2014). Cardiovascular disease is an important cause of mortality and morbidity in India. Data from the Registrar General of India show that CVD ranks first among the top five causes of deaths in India that was classified in different populations (urban vs rural, developed vs economically backward states, women vs men, and at middle aged vs all-ages) (Gupta et al., 2012).

There are many risk factors coupled with coronary heart disease and stroke. Some risk factors such as ethnicity, family history and age, cannot be altered. Other peril factors that can be treated or changed include high cholesterol, tobacco exposure and alcohol consumption, hypertension, rheumatoid arthritis (Deodhar et al., 2016), sedentary lifestyle, diabetes, periodontitis (Joo et al., 2016), and unhealthy diets. There is also an urge to determine the “causes of the peril factors” (Gupta et al., 2012). Triglyceride-rich lipoprotein cholesterol is considered as a perpetual target, reported by mendelian randomisation analysis (Najam and ray, 2016). Although there is no definite
evidence from trials for aiming non-LDL lipoproteins, mendelian randomisation studies have demonstrated that spotting atherogenic triglyceride-rich lipoproteins and their remnants may provide benefit (Varbo et al., 2015).

Prevention of cardiovascular diseases (CVDs), specifically atherosclerosis, remains a top concern on the global health agenda (Sarrafzadegan and Gotay, 2015). Atherosclerosis is an inflammatory arterial disease of the wall of large and medium sized arteries that is hastened by enhanced levels of low density lipoprotein (LDL) cholesterol in the blood.

Figure 1. Immune and inflammatory cells in atherosclerosis

Atherosclerotic lesion and the relatively unaffected areas are depicted in Fig.1. The endothelial cells above the lesion area are polygonal in shape, whereas normal endothelial cells are aligned with the direction of flow. Usually the intima is so thin, but it is greatly expanded in the lesion area, where it contains vascular dendritic cells (DCs), macrophages, and foam cells as well as occasional T lymphocytes. The foam
cells surround the necrotic core, which is thought to be composed of foam cells that have undergone secondary necrosis. The normal media is populated by smooth muscle cells that are organized by several elastic laminae. The normal adventitia is populated by sparse T cells, B cells and other lymphocytes as well as vascular DCs. In the lesion area, the lymphocytes organize into tertiary lymphoid structures, probably containing high endothelial venules and other vessels. The angiogenic process eventually leads to neo-vessels invading the intima, a process that is thought to destabilize plaque and precipitate rupture events. The stages of atherosclerotic disease progression are summarised in the flowchart given below.

LDL particles trapped in an artery can undergo oxidation and be taken up by macrophages as directed by scavenger receptors on the cell surfaces (Ridker and Morrow, 2003; Nishida et al., 2016). The lipid peroxides formed due to the espousal of oxidised LDL by macrophage assist in the aggregation of cholesterol esters, which leads to the formation of foam cells (Ross, 1999; Escate et al., 2016). The extent to which LDL is altered can differ considerably. Once altered and espoused by macrophages, LDL stimulates the foam cells. Eviction and seclusion of modified LDL are the vital parts of the initial, preventive aspect in inflammation and diminish the causes of
modified LDL on smooth muscle and endothelial cells (Ross, 1999). More and more monocytes are enticed to modified LDL which act as chemotactic and can cause robust inflammatory reaction by triggering the replication of activated macrophages and the entry of nascent monocytes into lesions. LDL particles rely upon for its atherogenicity to a degree of its ability to be altered (Tomkin and Owens, 2012). Oxidatively modified LDL (OxyLDL) induces a myriad of cellular responses which contribute to vascular dysfunction. There are overwhelming evidences which suggest that increased and/or modified LDL is a major risk factor for atherosclerosis (Galkina and Ley, 2009).

1.1 Oxidised LDL (OxyLDL)

OxyLDL is generally called as the key player in atherogenesis and it could be a possible biomarker for CVD. The piled-up data show that low levels of OxyLDL can be atheroprotective via activation of reverse cholesterol mechanism and cytoprotection. Recent studies revealed that OxyLDL does exist in atherosclerotic lesion as well as in circulation (measured by sandwich ELISA procedure with an anti-OxyLDL monoclonal antibody) (Itabe et al., 1996). According to the oxidation hypothesis of atherosclerosis, OxyLDL plays a pivotal role through the induction of foam cell formation, alteration of nitric oxide signaling, initiation of endothelial activation, and expression of adhesion molecules that accelerate leukocyte homing to the site of atherosclerosis (Galkina and Ley, 2009). The important point to be considered is that the OxyLDL studies from various laboratories showed variegated results because of the diversified preparations. There is no accepted ‘gold standard’ for preparing OxyLDL, so it cannot be defined or characterised (Levitan et al., 2010).
1.2 MONOCYTE/MACROPHAGE

Immune cells (monocytes) are formed in the bone marrow and traffic via bloodstream to peripheral tissues in the body where they become macrophages (Shi and Pamer, 2011). Macrophages were the first inflammatory cells to be associated with atherosclerosis. In their ground-breaking paper, Gerrity and coworkers (1979) identified macrophage as the vital component of the atherosclerotic plaque in porcine specimens. The monocyte/macrophage is a critical cell in the pathogenesis of atherosclerosis, capable of secreting many factors, such as chemokines, cytokines, growth factors, and reactive oxygen species, which lead to lesion development (Mary et al., 2012). The ability of macrophages to produce cytokines, growth factors, proteolytic enzymes may play a vital role in cell damage and repair that start to happen in lesion progression (Ross, 1999; Sprague and Khalil, 2009).

THP-1 cells are appropriate for the study of the novel functions and mechanisms involved in monocytes–macrophages in the cardiovascular system (Qin, 2012). CD14 a 55 kDa glycoprotein with multiple leucine-rich repeats, is widely used as a monocyte/macrophage marker, because of its abundance on these cells (Setoguchi et al., 1989). CD36 is an ~85 kDa multipass transmembrane glycoprotein and is one of the major receptors for oxidized low density lipoproteins belonging to macrophage scavenger receptor class B (Helming et al., 2009). CD68 is a 110 kDa glycoprotein highly expressed by classical macrophages and it is also a member of the lysosome associated membrane protein (Kunz-Schughart et al., 2003). MAC387 is a 14 kDa macrophage differentiation marker found in plaque and inflammatory lesions (Stoneman et al., 2007).
1.3 Inflammatory Mediators

Inflammation plays a key role in the pathogenesis of atherosclerosis (Liehn et al., 2006). Inflammatory mediators released by the monocyte/macrophage, smooth muscle cells and endothelial cells may exacerbate atherosclerosis by direct or indirect mechanisms (Hartman and Frishman, 2014). The inflammatory mediators MCP-1, COX-2, TNF-α, IL-6 and NFκB, together with inflammatory cells, are important in the inflammation cascade. Monocyte chemoattractant protein-1 (MCP-1), referred as chemokine (C-C motif) ligand-2, plays a fundamental role in monocyte recruitment and in the pathogenesis of atherosclerosis (Aiello et al., 1999). Cyclooxygenase (COX) 2 is expressed in atherosclerotic lesions and its genetic deletion from hematopoietic cells reduced atherosclerosis by 51% (Burleigh et al., 2005). TNF-α is a proinflammatory cytokine involved in the production of IL-6 by endothelial cells which plays a part in the pathogenesis of atherosclerosis (Bruunsgaard et al., 2000). OxyLDL enhances the expression of upstream proinflammatory cytokine IL-6, which propagates the downstream inflammatory cytokine responsible for atherosclerosis (Schieffer et al., 2004; Hartman and Frishman, 2014). NFκB regulates the expression of many genes involved in the induction and progression of atherosclerotic lesions, chemokines (e.g., MCP-1) and cytokines (e.g., TNF-α, IL-6) (Mittal et al., 2014).

NFκB may not only contribute to the different stages of atherosclerosis development, but also contributes to cell type-dependent, regulation of various genes (e.g., macrophages versus endothelial cells) (De Winther et al., 2005). Differentiation of the human monocytic cell line THP-1 into macrophage-like cells was induced by exposure of the cells to phorbol myristate acetate. NFκB is thought to be one of the more important nuclear factors in mammalian cells. Several stimulants such as LPS,
superantigen, phorbol esters, and cytokines are known to induce the appearance of NFκB in the cytoplasm and its translocation into the nucleus.

1.4 Notch Signaling pathway

Notch signaling is a complex juxtacrine pathway, highly conserved through evolution (illustrated in Fig.2). Both the Notch receptor (Notch1-4) and its ligands (Jagged – Jag1, Jag2 and Delta-like - DLL1, DLL3 and DLL4) are transmembrane proteins (Fung et al., 2004; Nakano et al., 2016). Binding of ligands to Notch receptors results in two proteolytic cleavage events in the Notch receptor, first by ADAM-family metalloproteases and the second by γ-secretase, an enzyme complex that contains presenilin, nicastrin, PEN2 and APH1. The second cleavage releases the Notch intracellular domain (NICD) into the cytoplasm (Geling et al., 2002). NICD then translocates to the nucleus and forms a complex with the DNA-binding protein CSL (CBF1, Su(H) and LAG-1) and its coactivator Mastermind (Mam) p300 proteins. Notch/ CSL-dependent transcription results in the expression of target genes Hes1-Hes7 (hairy/enhancer of split) and Hes related (Hey1-2 and Hey L) genes. Notch signaling is naturally downregulated in adult compared to embryonic life (Andersson et al., 2011). In agreement with many embryonic proteins, levels of Notch1 and Hes1 drop in postnatal heart, after birth. Notch ligands and receptors get upregulated in damaged and regenerating tissues, including heart, as well as in vessels (Quillard and Charreau, 2013). Enhancement of Notch activation after myocardial infarction in the adult increases survival rate and improves cardiac function (Kratsios et al., 2010). The role of Notch signaling in atherosclerosis cannot be complete without analysing the key role of Notch in macrophages, which trigger the inflammatory response and subsequent plaque formation in atherosclerosis (Rusanescu et al., 2008).
Multiple Notch receptors and ligands are expressed in human macrophages (Fung et al., 2007). Activated macrophages interact with, and stimulate the proliferation of adjacent vascular smooth muscle cells (VSMCs) through secreted inflammatory molecules as well as direct contact via Notch pathway (Rusanescu et al., 2008). Particularly, the receptor Notch1 and its ligand Jagged1 are the potent players of the Notch family and their expression on the surface of cardiomyocytes activates Notch pathway in the heart in response to stress (Quillard and Charreau, 2013).

Collective data arise from the concept that Notch pathway is important for inflammatory events involved in cardiovascular pathogenesis, and that it may serve as a new target for therapeutic approaches (Marchant et al., 2012). Communication and cellular adaptation between vascular, cardiac and immune cells and role of Notch signaling in the control of inflammatory reaction is illustrated in Figure 3.
Figure 3. Notch signaling, Inflammation and Atherosclerosis

1.7 **CELL SHAPE AND MACROPHAGE POLARISATION AS M1 AND M2**

The word ‘macrophage’ is derived from the Greek ‘makros’ means ‘large’, and ‘phagein’ means ‘eater’. Macrophages are notably phagocytic, as discovered by Elie Mechnikoff in 1882 (Swirski et al., 2016). Charles Dudley Mills, (2000) named the macrophages as M1/kill and M2/repair. Macrophages were viewed as “trash disposal unit” (Nathan, 2012), “adaptive dictator” (Mills, 2012) and also as an “enigma”, but the macrophages do stupendous performance in tumor, polarizing as M1 (inhibit proliferation) and M2 (promote proliferation) in response to local stimuli (Mills, 2015). Macrophages are central regulators in innate immunity and are implicated in a variety of immune functions, including host defense (M1) and wound healing (M2) (McWhorter et al., 2013). Macrophages exhibit phenomenal plasticity and can alter their physiology in response to environmental cues. They are highly multifarious cells that can quickly change their function in response to local microenvironmental signals (Kierdorf and Dionne, 2016). Different macrophage population with distinct functions can occur during these signals (Mosser and Edwards, 2008). Interpretation of the
scientific findings suggested that embryonic macrophages are inherently reparative while monocyte-derived macrophages are inherently inflammatory (Swirski et al., 2016). In an effort to simulate the T-cell literature, macrophages have been classified along with what could be viewed as a direct scale, on which M1 macrophages represent one extreme as pro-inflammatory (Classically activated macrophages) and M2 macrophages represent the other extreme as anti-inflammatory (Alternatively activated macrophages (Martino et al., 2002). It seems very curious to contemplate that a related ‘switch’ in macrophage morphology may also occur during atherosclerosis. Studies will feasibly need to target on macrophage heterogeneity distinguished by differential expression of different molecules or molecular networks that have functional importance for atherogenesis rather than heterogeneity based on the aforementioned M1 vs. M2 paradigm (Kadl et al., 2010).

1.8 Chemokines

Substantial evidence from clinical and experimental research suggests that chemokines and chemokine receptors play critical roles in directing leukocytes into atherosclerosis-prone vessels. Taking into account the chemokine abundance within atherosclerosis-prone arteries and the variety of chemokine receptors on leukocytes, it is clear that a tightly controlled network regulates recruitment, retention, and emigration of leukocytes in the arterial wall (Galkina and Ley, 2009).

Cytokines and chemokines are key players during acute and chronic inflammation. The regulation of chemokine production depends on many factors and is tightly regulated during inflammation. Many cytokines, such as TNF-α, IL-1, IL-2, IL-3, IL-6, CXCL8, IL-10, IL-12, IL-15, IL-18, IFN-γ, M-CSF, TGF-β1, TGF-β2, and TGF-β3, are detected within atherosclerosis-prone vessels (Canault et al., 2008). Under conditions of hyperlipidaemia, macrophages produce TNF-α, IL-1, IL-6, IL-12, IL-15
and IL-18 but also the anti-inflammatory cytokines IL-10 and TGF-β. The chemokine/chemokine receptor network is essential for direction of leukocyte migration in homeostatic and inflammatory conditions (Tedgui and Mallat, 2006).

CCL2 was the first chemokine shown to affect atherosclerosis. CXCL4 can induce activation of ECs by inducing expression of E-selectin, NFκB activation, and enhanced binding of oxLDL to ECs (Serbina and Pamer, 2006). Recently, a unique role for chemokines was documented in the shear stress–dependent modulation of atherosclerotic lesion composition (Cheng et al., 2007). The cytokine MIF is produced by ECs, SMCs, and macrophages in early and advanced atherosclerotic lesions. Binding of MIF to its newly discovered receptor complex of CXCR2 and CD74 resulted in elevated monocyte arrest on atherosclerotic endothelium (Bernhagen et al., 2007). CXCL10 is a potent mitogenic and chemotactic factor for SMCs and can modulate the local balance of the effector and regulatory arms of the immune system through the reduction of Tregs in aorta (Heller et al., 2006). Other chemokines, including CCL3, CCL4, CCL11 and CXCL12, are expressed in human and mouse atherosclerotic aorta, but the role of these chemokines in atherosclerosis remains unclear (Kraaijeveld et al., 2007; Galkina and Ley, 2009).

1.9 ANTIBIOTICS

Antibiotic drugs have reduced the burden of common infectious diseases and become essential for many medical interventions against life-threatening diseases (Van Boeckel et al., 2014). On the other hand, overuse of antibiotics can be disastrous in the long run. Hence, judicious use of antibiotics is required, but acceptable strategies to achieve this goal and to address the challenges must be devised and communicated. Sustained efforts will be required to educate and re-educate physicians about the long-
term consequences of antibiotic overuse. Here, technical issues need to be highlighted so that the physicians understand and appreciate the message.

**Figure 4. Antibiotic purchase and cause of death**

Outpatient antibiotic purchases from retail outlets in India had doubled in 2009 when compared to 2005 (Fig.4a). Cardiovascular diseases hold a major part when compared to other disease(Fig.4b). Antibiotics have led to an extraordinary decrease in morbidity and mortality associated with bacterial infections. Yet, despite the great benefits, antibiotic use has been linked to various adverse side effects, including mitochondrial dysfunction and oxidative damage via reactive oxygen species (ROS). Extensive production of ROS has been implicated in atherosclerosis by inducing the
chronic activation of the vascular endothelium and components of the immune system (Kalghatgi et al., 2013).

1.10 Diosgenin

Historically, all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the complex form of crude extract, mixture etc. Today, a substantial number of drugs are developed from plants (Binesh and Gnanam, 2009). Diosgenin is a member of the steroidal sapogenin family, found in several plants including Solanum, Trigonella and Dioscorea species. Steroidal saponins have been reported to have a variety of medicinal uses. Structure of diosgenin is depicted in Fig.5.

![Molecular structure of Diosgenin](image)

Figure 5. Molecular structure of Diosgenin

Earlier investigations have shown that diosgenin plays an important pharmacological role as an antidiabetic agent (Ribes et al., 1986), an antioxidant (Rao, 1996), a plasma cholesterol lowering agent (Ravikumar and Anuradha, 1999), an antineoplastic and anti-inflammatory agent (Yamada et al., 1997; Sur et al., 2001), and as an anti-metastatic agent (Chen et al., 2011). It is also divulged as a multitarget based chemopreventive or therapeutic agent against several cancer types (Raju and Mehta, 2009). So, in this study, the anti-atherosclerotic role of diosgenin was investigated.