7. PROTECTIVE EFFECT OF FERULIC ACID AND METHANOLIC FRACTIONS OF *Terminalia arjuna* SEED EXTRACT ON IMMUNOHISTOCHEMISTRY OF COX-2 PROTEIN EXPRESSION IN HEART TISSUE OF MERCURY INTOXICATED RATS

7.1 Introduction

Now a days most of the researchers interest focus on the understanding of the toxic effects of mercury on the cardiovascular system and its association with hypertension, carotid atherosclerosis, myocardial infarction and coronary heart disease (Wiggers *et al.*, 2008). However, mercury chloride accumulation and its toxicity have caused to cardiac dysfunction leading to heart failure during chronic period (Vijayakumar *et al.*, 2014).

Inflammation is traditionally defined as a local reaction of heart tissue to irritation, injury, swelling, pain, and loss of function. However, it is now viewed in terms of the activation of cells of the innate immune system, the coordinated actions of the mediators they produce, and altered inflammatory protein expression. Heart dysfunction is mainly associated with an inflammatory reaction, which is a prerequisite for healing and scar formation during myocardial infarction (Frangogiannis *et al.*, 1998). Reactive oxygen species have the potential to directly injure cardiac myocytes and vascular cells and may be involved in triggering inflammatory cascades through the induction of cytokines (Dhalla *et al.*, 2000). Therefore, inflammatory response and cytokine elaboration are integral components of the host response to tissue injury and plays a particularly active role after infarction. The degree of the inflammatory response in turn is an important
determinant of the host’s outcome. Cytokines are released by the host myocardium to modulate tissue repair and adaptation after injury (Nian et al., 2004). Therefore, heart damage is followed by an inflammatory reaction which plays a key role in all stages of repair after heart tissue damages. However, inflammatory reaction may as well contribute to myocardial damage, remodeling, and development of congestive heart failure after heart tissue damages (Hansen, 1995; Zidar et al., 2005). Prostaglandins synthesized via the COX-2 pathway are important mediators in inflammation after heart tissue damage (Saito et al., 2000; Scheuren et al., 2002; LaPointe et al., 2004).

Cyclooxygenases COX is a rate-limiting enzyme which catalyzes the first step in the synthesis of prostanoids (prostaglandin and thromboxane). COX is present in at least two isoforms, COX-1 and COX-2, which catalyse identical reaction. Therefore, COX-1 is constitutively expressed in many cells and tissues. COX-2 is normally not present in most cells, its expression can be induced by a wide variety of stimuli including inflammatory mediators and growth factors (Hinz and Brune, 2002; Simmons et al., 2004). Hence, COX-2 expression is induced by various cytokines, growth factors, lipopolysaccharides (LPS) (Sonis et al., 2004). A consequence of COX-2 involvement and production of prostaglandins is the characteristic edema and tissue injury present in inflammatory diseases (Kuwano et al., 2004). The role of prostaglandins and their free radical catalyzed isomers in the modulation of cardiac function is poorly understood. However, cyclooxygenase (COX)-2 is upregulated in animal models of cardiac failure and its expression has been detected in cardiomyocytes in heart failure in humans. Deletion of the COX-2 gene may result in myocardial fibrosis. With this point of view, the present
experimental study has been designed to determine the cardiac productive role of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract against mercury chloride intoxicated rats through the COX-2 protein expression. Therefore the present experimental study sought to determine expression of COX-2 in the failing heart by immunohistochemistry.

7.2 Observation

7.2.1 Heart Tissue

7.2.1.1 Untreated Control

The negative expression of COX-2 observed in the untreated control group rat. Fig. C1 and C2 shows negative expression of COX-2 protein and also cardiac muscle fibers were found to be of uniform size, shaped and regular arrangement of cardiac segment is normal appearance of myofibrils.

7.2.1.2 Mercuric chloride (HgCl₂) treated groups

At sub lethal dose of mercuric chloride treatment, the intoxicated heart tissue shows the inflammation and abundant amount of positive expression of COX-2 protein. Fig. C3 and C4 shows the irregular shape of nucleus and focal lesions in many areas and fragmentation of muscle fibres with confluent retrogressive lesions, hyaline necrosis, and edema were observed in positive expression of the heart tissue.

7.2.1.3 Mercuric chloride followed by Ferulic acid treated groups

Fig. C5 and C6 show the mild expression of COX-2 protein expression in the myocardial tissue. The size and shape of the cardiomyocytes, myocardial cells and myofibrils are restored to near normal level.
7.2.1.4 Mercuric chloride followed by methanolic fractions of *Terminalia arjuna* seed extract treated groups

Fig. C7 and C8 also show the mild expression of COX-2 protein in the nucleus of myocardial cells. The size and shape of the cardiomyocytes, myocardial cells and myofibrils are restored to near normal level.

7.2.1.5 Ferulic acid alone treated groups

Fig. C9 and C10 Show the alone treatment of restoration of normal heart tissue with cardiac myocytes. The negative COX-2 protein expression was noticed in Ferulic acid alone treated heart tissue.

7.2.1.6 Methanolic fractions of *Terminalia arjuna* seed extract alone treated groups

Fig. C11 and C12 Show the alone treatment of restoration of normal heart tissue with cardiac myocytes. The negative COX-2 protein expression was noticed in methanolic fractions of *Terminalia arjuna* seed extract alone treated heart tissue.
7.3 Discussion

Cyclooxygenase (COX) is a key enzyme required for the conversion of arachidonic acid to prostaglandins. The two known COX isoforms are referred to as COX-1 and COX-2. The metabolism of arachidonic acid by the cyclooxygenase (COX) pathway generates eicosanoids, which have been implicated in the pathogenesis of a variety of human diseases, including heart disease (Matsuyama et al., 2010). COX is the first enzyme in the pathway responsible for producing prostaglandins and thromboxane from arachidonic acid. COX-2 is expressed momentarily and strongly on growth factors, promoters and certain endotoxins, and is involved in inflammation, cell growth and differentiation. COX-2 is induced in response to pro-inflammatory cytokines and stress (Matsuyama and Yoshimura, 2004). The proinflammatory role appears to be mediated mainly by COX-2. Myocardial inflammation constitutes a major component of the pathologic changes observed during mercury intoxication. Prostaglandins (PGs) are lipid mediators which contribute to the vasodialation, edema and plasma protein leakage that occur during the inflammatory response (Yang et al., 2000). In the present experimental study, the level of TNF-alpha expression was drastically increased in mercury intoxicated heart tissue (vide in chapter 8). This result suggested that an enhanced level of oxidant properties leads to formation of TNF-alpha expression (inflammatory response).

Myocardium inflammation (leukocyte infiltration and interstitial edema) along with contractile dysfunction and death of cardiac muscle cells are hallmarks of acute cardiac failure. Along with cytokines, histamine and kinins, the
Eicosanoids are a family of lipid mediators that contribute to the vasodilation and leakage of plasma proteins (vide in Chapter 5) and fluid into the interstitial space, which occurs during the acute inflammatory response in the myocardium and in other tissues (vide in chapter 3). In cardiovascular diseases, COX-2 is known to have a cardioprotective protection effect that alleviates ischemia/reperfusion injury. COX-2 is well-established to participate in prostanoid production and is implicated in the processes of disease and expressed in inflammatory sites that often have harmful effects. It is believed that COX-2 is induced during both acute and chronic inflammatory responses, and is primarily responsible for the prostaglandins synthesis that ensues. The prostaglandins in particular play a role in the pathogenesis of inflammation involving cell-mediated immune responses.

In normal animal, COX-2 protein present in the several cells of various organs. Particularly it is abundantly present in cardiac cells and appears to protect against inflammatory promote cell growth. COX-2 is expressed in areas of infarction in the heart tissue (Wong et al., 1998). The extracellular matrix (ECM) forms a complex meshwork composed of structural proteins which are responsible for tissue strength and structure. Matricellular proteins are non-structural proteins with a minimal expression in the normal heart. However, their expression increases after injury, where they modulate cell function and behavior through regulation of cell–cell and cell–matrix interactions (Spinale, 2007). Cardiomyocytes only expression of COX-1 under normal circumstances, but COX-2 was induced on exposure to free radicals. Here, the present experimental study shows that mercuric chloride induces COX-2 in the adult rat heart when administered exogenously and enhances the free radical generation, the principal product generated in cardiac cells. The isoform responsible for the increased product formation was assessed using specific inhibitors of COX-2 isoforms (Smith et al., 1998).
The present experimental result clearly demonstrates for the first time induction of COX-2 and activation of TNF-alpha and TGF-β1 in the failing cardiac tissue in the mercury intoxicated rats. Fig. C3 and C4 showed abundant expression of COX-2 expression in damaged heart tissues. In contrast, expression of the enzyme in dilated cardiomyopathy was only seen in areas of myocardial fibrosis. Induction of COX-2 molecules was rarely seen in normal control cardiac tissue. These observation suggested that an increased expression of COX-2 in the failing myocardium and suggest that induction of COX-2 increased and formation of prostanoids may contribute to the pathophysiology of heart failure. The mercury toxicity induces synthesis of the locally secreted growth factor transforming growth factor β1 (TGF-β1) in cardiac fibroblasts and myocytes (vide in chapter 9). Interaction of TGF-β1 with its receptor causes expression of ECM proteins, collagen synthesis, and proliferation of cardiac fibroblasts (Villarreal et al. 1996; Tomasek et al. 2002). In cardiovascular diseases, COX-2 is known to have a cardioprotective protection effect that alleviates injury (Bolli et al., 2002). COX-2 is induced by inflammatory cytokines such as TNF-α, which is produced in heart failure (Torre-Amione et al., 1996). In addition to cytokines, hypoxia, an important feature of ischemic myocardium, induces COX-2 (Schmedtje et al., 1997). Wang et al. (1998) reported that abundant expression of COX-2 in myocytes of infarcted myocardium. The present experimental study also observed up regulation of COX-2 protein expression in mercuric chloride intoxicated rats.
Little is known about the expression and regulation of COX-2 in the myocardial infarction. The accumulation of mercuric chloride induced COX-2 expression in the myocytes and inflammatory cells of rat with heart failure. There are several other mediators known to induce COX-2 in other cells that may contribute to the induction of the enzyme in myocytes of failing heart tissues. For example, hypoxia and tumor necrosis factor-α, important features of infarcted heart dysfunction. The pathway of COX-2 induction and the subsequent formation of prostanoids involve activation of TNF-α. The histoarchitecture of immunohistochemistry of heart tissue suggest that the induction of COX-2 and TNF-α from the cytoplasm to the nuclei in cardiomyocytes and inflammatory cells of the mercury intoxicated cardiac tissue. The present studies suggest that ischemia and inflammatory mediators are responsible for activation of TNF-α and induction of COX-2 in this condition.

During the recovery period, administration of a Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract against cardiac protection in mercury intoxicated heart tissue. The obtained results indicated that COX-2 inhibition reduced collagen deposition in the heart tissues after myocardial infarction. This beneficial alteration in the remodeling process probably reduced cardiac tissue stiffness, thus accounting for the improved cardiac function (Vide in chapter 6). The decrease in collagen likely resulted from decreased TGF-β1 release by macrophages, myocytes, or fibroblasts, as detected less TGF-β1 expression in mercury treated hearts (Vide in chapter 9). Recently this result is consistent with reports using two different models of cardiac injury in the rats. In these studies, chronic COX-2 inhibition reduced the extracellular matrix, TGF-β1 production,
cardiac injury, thus improving cardiac function in the animal. Therefore, the decrease in collagen content (not observed directly) might be resulted from decreased proliferation of fibroblasts and decreased infiltration of inflammatory cells, which synthesize and release cytokines and growth factors involved in extracellular matrix formation. A better understanding of the role of COX-2 in inflammation led to drug discovery programs aimed at identifying new anti-inflammatory agents that selectively inhibit COX-2 activity (Penning et al., 1997). COX-2 is thought to be involved in the inflammatory process. Inhibition of its activity achieves the same therapeutic effect. The result suggests that administration of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract reduced the mercury toxicity induced COX-2 upregulation in the heart at all time points. Although the effects of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on COX-2 expression implicate the participation of the protective receptor which is involved in the repairing of damaged or diseased heart tissue. The present experimental pathological findings confirmed the biochemical data and showed that mercuric chloride accumulation caused cardiac damage in the form of myocardial necrosis, interstitial hemorrhage, mononuclear cell infiltration and fibrosis. Mercuric chloride increased the cardiac tissues COX-2 expressions. However, administration of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract reduced the cardiac histo pathologic lesions, preserved its structure and decreased the COX-2 expression in mercuric chloride followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract groups respectively.