8 PROTECTIVE EFFECT OF FERULIC ACID AND METHANOLIC FRACTIONS OF *Terminalia arjuna* SEED EXTRACT ON TNF-α PROTEIN EXPRESSION OF HEART TISSUE IN MERCURY INTOXICATED RATS

8.1 Introduction

Causative factor of cardiovascular disease is the number one disease in human beings. Number of peoples are affected by cardiovascular disease to causes death in Asian country (Vijayakumar *et al.*, 2014). Heavy metal exposure especially mercury and its compounds have been linked to increased incidence of cardiovascular diseases in animals (Patrick, 2003; Bhatnagar, 2006). Consumption of high quantity of mercury-contaminated fish changes the blood pressure and cardiac autonomic activity in human beings (Valera *et al.*, 2011). Therefore, most serious effect of heavy metal toxicity mercuric chloride poisoning is damage to the heart and promotes the cardiovascular disease.

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine with cardio depressant properties. An elevated plasma level of TNF-α may be found in dilated cardiomyopathy, in end-stage ischaemic heart disease. In normal condition the peripheral blood mononuclear cells are the main source of TNF-α. A positive correlation is found between the severity of heart failure and TNF-α level in number of animals. Another reason for elevated TNF-α level in the cardiac system is mainly dependent on the excessive catecholamine production and generalized endothelial dysfunction.
Most of the investigators noted that tumor necrosis factor-alpha (TNF-α) proinflammatory cytokine with potent negative inotropic effects, is established in heart failure. An enhanced level of TNF-α suggests that it may play a much broader pathophysiologic role in heart failure. Although the exact clinical significance of elevated levels of TNF-α in advanced heart failure is uncertain, it is clear that elevated levels of TNF-α can produce a number of the classical features of heart failure.

The biological functions of TNF-α is varied and the mechanism of action is somewhat complex. This protein, conferring resistance to certain type of infections on the one hand and causing pathological complications on the other, carries out contradictory roles (Feldman et al., 2000). TNF-α plays several therapeutic roles within the body, which include immune stimulation, resistance to infection agents, resistance to tumors (Vilcek and Lee, 1991), sleep regulation (Krueger et al., 2001), and embryonic development (Wride and Sanders, 1998). However, the major role of TNF-α seems to be as an important mediator in resistance against such infections. TNF-α may contribute towards resistance of infection through activation of neutrophils and platelets, enhancement of macrophage/NK cell killing abilities, and stimulation of the immune system (Feldman et al., 2000). Over production of TNF-α can induce necrotic or apoptotic cell death (Beyaert and Fiers, 1994). Necrosis is characteristic with cell swelling, organelle destruction, and cell lysis.

Highly mercury toxicity level can cause reactive oxygen species (ROS) production and expression of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) in many cell types and particularly in heart tissue. The
generation of ROS may partly explain how hyperglycemia mediates cardiac tissue damage and remodeling. The ROS is known to trigger TNF-α gene expression and its signaling pathway (Aikawa et al., 2002; Quan et al., 2011). Several studies have shown that high toxicity induces high TNF-α expression, which significantly induces apoptosis in cardiomyocytes (Kageyama et al., 2011; Min et al., 2009). With this point of view, the present experimental study has been designed to determine the level of TNF-α expression in the heart tissue of treated animals.

8.2 Observation

Fig. 17 and 18 shows the tumor necrosis factor-alpha (TNF-α) protein expression is identified in the heart tissue of both control and treated animal by using western blot analysis. During the mercuric chloride treatment, the level of TNF-α protein expression was drastically increased in the rat heart tissue when compared to the normal. During the recovery period, mercuric chloride followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract, an enhanced level of TNF-α expression significantly decreased. Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract alone treatment showed maintaining near normal level of TNF-α protein expression.
6.3 Discussion

At high level of heavy metal exposure to the animals is well known, but low levels of continuous exposure of heavy metals may lead to chronic adverse health effects (Jarup, 2003). The severity of adverse health effects is related to the chemical form of heavy metals and it also depends upon the level of dose and its duration. Although they have no known metabolic function, when present in the body heavy metals disrupt normal cellular processes, leading to toxicity in a number of organs (Alissa and Ferns, 2011). They are relatively poorly absorbed into the body, but once absorbed are slowly excreted and accumulate in the body causing organ damage. In additions heavy metals contribution to cardiovascular disease is still incompletely understood. Heavy metal intoxication mechanism was not clearly understood. Among the heavy metals, mercury and its compounds induced oxidative damage have been observed \textit{in vivo} in the present experimental work particularly in myocardial tissues. The mechanisms by which mercury exerts its cardiovascular effects are not fully understood.

Cardio-toxicity was induced by mercuric chloride to promoting cell apoptosis and oxidative stress damage reflected as depressed cell viability and cytokines and increased cell apoptosis in heart tissue. The ROS are iniquitous, highly diffusible molecules that are increased during inflammation presses in cytokines. Activated phagocytes, neutrophils and macrophages recruited to site of inflammation and also act as potent sources of ROS production (\textit{vide} in Chapter 4). An excess of ROS \textit{in vivo} can adversely alter intracellular reduction /oxidation homeostasis and ROS have been implicated as a major cause of cellular and tissue
damage associated with chronic inflammation (Coopar et al., 2002). Exposure to mercuric chloride resulted in a significant increased in the production of the pro-inflammation cytokine TNF-α which are mediators of the inflammatory response and which are released from different cell types (Sainte-marie et al., 2007). TNF-α is responsible for cardiac injury. Excessive amount of TNF-α expression has also been implicated in the pathogenesis of many chronic inflammatory diseases (Firestein et al., 1994). Mercury-induced redox imbalance may be caused by either increased reactive oxygen species generation or by reduced antioxidants defense capacity (vide in chapter 4). This is supported by observations that both enzymatic and nonenzymatic antioxidants and bio-markers (vide in chapter 4 & 5).

The first recognition that TNF alpha might participate in the development of congestive heart failure (CHF) came in 1990 when Levine et al. demonstrated that circulating levels of TNF alpha were elevated in patients with end stage heart failure and cachexia. Tumor necrosis factor alpha (TNF-α expression), a protein belonging to the family of cytokines, is one of the leading mediators of the immune response to inflammation. Its widespread biological effects are modulated by two circulating binding proteins corresponding to the extracellular domain of the membrane receptors, namely soluble TNF receptors. TNF-α was first supposed to be linked with congestive heart failure. TNF-α expression exerts a negative inotropic effect (Amione et al., 1996). Moreover, TNF-α expression has been suggested to trigger the apoptotic process in cardiac myocytes. How-ever, not only does TNF alpha have immediate negative inotropic properties, but it can recapitulate the cellular and biochemical abnormalities that characterize the failing heart.
An enhancement in the level of TNF-α expression in the mercury intoxicated heart tissue may result in further progression of heart failure. The present experimental study covered various components of the heart failure through the biological significance of the disturbed TNF-α expression system. An enhancement of TNF-α expression was mainly caused by continuous exposure of mercury administration in rats due to the enhancement of biologically active soluble TNF receptors in mercury intoxicated heart tissue. The pathophysiology of mercury induced by cardiotoxicity is multifactorial and complex, but the main mechanisms are increased damage from oxidative stress (Li et al., 1999). TNF-alpha is generally considered to be harmful (Murray and Freeman, 1996). TNF-alpha may, therefore, have different effects depending on its mode of secretion, the species and type of cell, and the nature of the stimulus (Haudek et al., 2001). Recent studies have focused their attention on the role of the proinflammatory cytokine tumor necrosis factor (TNF) in the development of heart failure. The normal heart does not express TNF; however, the failing heart produces high quantity. It is a direct relationship between the level of TNF-α expression and the severity of disease. In addition, in the present experimental study clearly demonstrates the TNF effects at cellular, biochemical and bioenzymological changes in mercury intoxicated rats with congestive heart failure.

The cellular effects of TNF alpha are highly pleiotropic. Tumor necrosis factor alpha enhances inflammation (Feldman et al., 2013). In the present experimental study, the inflammation was noticed in the histoarchitecture of the heart tissue (vide in Chapter 3). TNF alpha production exceeds the number of TNF alpha receptors located on the cell surface with excess TNF alpha being released into the circulation. Once released, TNF alpha exerts endocrine or exocrine effects
including initiation of metabolic wasting, microvascular coagulation, hypotension and fever. Tumor necrosis factor alpha has also been shown to have an important role in cell death through a variety of mechanisms including oxygen free radicals (Rahman, 2007). Excessive activation of TNF alpha in heart tissue expression leads to tissue necrosis and apoptosis. Our results clearly indicate that the production of TNF-α is sufficient to cause severe cardiac disease; however, the present experiments do not distinguish whether damage is caused by TNF-α directly, by inflammatory cells that have been recruited by TNF-α, by the expression of other cytokines, or by the induction of the generation of free radicals (Chen et al., 2008).

During the recovery treatment, Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat heart tissue showed decreased in the level of cytokine TNF-α protein expression to near normal level. The result suggested that TNF-α secretion is increased following chronic administration of mercuric chloride could promote the cardiac damages. It is suggest that protein expression of this cytokine may be regulated differentially during distinct phases of cardiac injury in rat. In addition, the present findings suggested that heavy metals, particularly mercury, interfere with the secretion of TNF-α offers a new mechanism to be considered in mercuric chloride induced cardiotoxicity. The present experimental study was, Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract administered rats, showed suppressed TNF-α expression, which could be established by the free radical scavenging potential of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract therapy preventing the depletion of reduced glutathione level in the heart tissue (vide in Chapter 4).
It is also clear how early antioxidant imbalance is reversed by Ferulic acid and methanolic fractions of *Terminalia arjuna*. The increase in TNF-α level is due to ROS generation, acts as an intracellular signal by changing the redox status of the cell. Hence, mercury intoxicated animals exhibit enhanced production of TNF-α, whereas Ferulic acid and methanolic fractions of *Terminalia arjuna* treated animals exhibit decreased TNF-α levels. This decrease may be due to inhibit the formation of free radicals and its cytotoxic effects. Administration of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract permeates the biological membrane and functions as an intracellular scavenger of free radicals. The present experimental work proves Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract prevents the cellular damage by its scavenging activity and thereby results in maintenance of intracellular and intra-organelle redox status (vide in Chapter 3). Our results are well in line with the observed moderate cardiac function in Ferulic acid and methanolic fractions of *Terminalia arjuna* supplemented rats. The administration of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatment, in conjunction with TNF-α administration, could minimize myocardial oxidative stress by either scavenging ROS or by decreasing ROS generation (vide in Chapter 4). The levels of TNF-α cytokines were significantly decreased following Ferulic acid and methanolic fractions of *Tarminalia arjuna* seed extract supplementation to mercuric chloride exposed rats. This might be due to the anti-inflammatory activity of Ferulic acid and methanolic fractions of *Tarminalia arjuna* seed extract. Our findings are the first evidence of cardioprotective effect of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract can reverse the decrease in TNF-alpha secretion that follows mercury exposure.