9. PROTECTIVE EFFECT OF FERULIC ACID AND METHANOLIC FRACTIONS OF *Terminalia arjuna* SEED EXTRACT ON TRANSFORMING GROWTH FACTOR (TGF)-β1 mRNA EXPRESSION OF HEART TISSUE IN MERCURY INTOXICATED RATS

9.1 Introduction

The mechanism involved in tissue damage in mercury intoxicated animals is the production of free radical generation through the oxidative stress (Bharathi *et al.*, 2014). Accumulation of oxidative stress promotes the inflammation in cell and tissues. Inflammation participates in defense against oxidative stress but also has the potential to injure heart tissues. Heart failure is also associated with an inflammatory reaction (Mehta and Li, 1999). Therefore, reactive oxygen species have the potential to directly injure cardiac myocytes and vascular cells and may be involved in triggering inflammatory process through the induction of cytokines (Nian *et al.*, 2004). Inflammatory response and cytokine elaboration are integral components of the host response to tissue injury and plays a particularly active role after heart failure.

Transforming growth factor beta (TGF-β) is a protein that controls proliferation, cellular differentiation, and other functions in a number of different organ systems. Five distinct isoforms of TGF-β have been identified and three of these, TGF-β1, TGF-β2 and TGF-β3 are found in all mammalian species but the form most implicated in cardiac fibrosis is TGF-β1. TGF-β1 is produced and activated in the cardiac tissue under stress conditions and plays a critical role in cardiac remodeling in response to cardiac stress (Wang *et al.*, 2002). However, over expression of TGF-β1 in heart tissue results in tissue fibrosis and organ
dysfunction. TGF-β1 is key mediators of cardiac hypertrophy and failure (Schnee, 2000; Cohn and Ferrari et al., 2000). The expression of TGF-β1 is mainly depend upon the expression of GAPDH mRNA in cells.

Changes in the gene expression of an organism are mainly responsible for transferring mechanical and hormonal stimuli through number of pathways. These changes in gene expression lead to heart failure. In the present experimental study is to identify a gene expression fingerprint for heart failure. “Housekeeping” genes are responsible for expressing the data of the level of quantitative gene expression levels of control. During the investigation period, the level of expression of the genes remains constant in the cells. This assumption is well documented, because housekeeping genes are of value in fully characterized systems. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data among the normal and treated cells. Number of investigators are measured the value of GAPDH as a housekeeping gene in tissues, the expression of GAPDH mRNA was measured in normal and treated tissue types. They are obtained the measurements through the quantitative real-time RT-PCR with specific target genes. Significant differences in the expression levels of GAPDH mRNA were observed between control and treated tissue of the same organ. Analysis of gene expression is playing a vital role at that time of treatment of a disease. It is very useful tool for biological research and detection of differential expression of a gene between normal and treated level. It can clearly provide novel idea for the treatment of disease or defect. The main advantage of quantitative real-time RT-PCR (Q-RT-PCR) techniques is very help to measure the gene expression in accurately in cells and tissues. During the measurement of gene expression the age and gender of the
species not influenced. Therefore, the levels of GAPDH mRNA expression in the tissues will provide standard data. These data confirm the variability of GAPDH mRNA expression previously indicated in published data and, as a result, may be used to define the variability of GAPDH expression between control and treated tissues. Furthermore, the obtained data can be used as a factor for the normalization of gene expression between tissues in experiments where GAPDH is being used as the experimental housekeeping control gene.

With this point of view, the present experimental study was initiated to investigate the ameliorative potential of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract against mercury induced cardiotoxicity intoxicated rats through studying genes expression in the TGF-β1 pathways.

**9.2 Observation**

Fig. 19 and 20 shows the mRNA in gene expression of RT-PCR both qualitative and quantitative analysis. TGF-β1 in gene expression of quantitative analysis made through the housekeeping genes (GAPDH) in the heart tissue of control and treated experimental rats. Mercuric chloride treated rats shows an two fold increased in the level of TGF-β1 gene expression. During the recovery period, the post treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract against mercuric chloride intoxication rats were shows the significant downward regulation of TGF-β1 gene expression. Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract alone treatment shows no response changes of TGF-β1 in gene expression at quantitative level of control rats.
9.3 Discussion

An association between mercury and its compounds exposure and an augmented risk for cardiovascular disease is an indication of increasing body burdens (Everett and Frithsen, 2008; Peters et al., 2010; Schutte et al., 2008). Heavy metals may be deposited in the heart muscle and produce cardiotoxicity even at low concentration of exposure to the animals (Limaye and Shaikh, 1999). The majority cases of myocarditis are due to heavy metal toxicity appeared in a significant proportion of heart failure (Caforio et al., 2012). Increasing evidence supports the role of free radicals related to the heart failure (Rahman et al., 2007). Most of the experimental works have shown that reactive oxygen species (ROS) plays a crucial role in the development of cardiovascular damages (Miller, 1998; Melinda Beck et al., 2004). Serum aminotransferase activities have long been regarded as indicators of tissue injury (vide in Chapter 5). In the present experimental study, administration of mercuric chloride promote the injury of heart tissue (vide in Chapter 3) alters the structure and function of myocardium in mercury intoxicated rats, leading to leakage of biomarker enzymes from the cells to the circulation (vide in chapter 5).

Quantification of mRNA abundance is playing a vital role for understanding the degree of pathological and compensatory mechanisms in heart failure. Quantitative real-time RT-PCR (RT-qPCR) technique has been used to findout the quantification of many gene transcripts in tissue sample. This instrument is more sensitive detection of weakly expressed gene transcripts in samples. Normally, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is most
frequently used reference genes in quantification of gene expression in tissue samples (Rebouças et al., 2013). In the present experimental study, the interpretation of changes in gene expression was dependent on GAPDH as reference gene. Through the gene normalization methods GAPDH Gene expression used in control and treated heart material. Single reference gene normalization compares both control and treated stages may be difficult because of the wide variation in expression at mercury toxicity effects. This effect was particularly pronounced for GAPDH, which would highly influence the interpretation of results.

The occurrences of cardiovascular damages or diseases mainly depend on the elevated level of TGF-β in experimental animals (Schmierer and Hill, 2007). TGF-β is a multifunctional and ubiquitously expressed growth factor. TGF-β families are markedly induced in the heart damages. Their families are playing a vital role in infarct healing and cardiac repair through their potent effects (Bujak and Frangogiannis, 2007). The availability of TGF-β isoforms stimulate the formation of extracellular matrix (ECM) in damaged heart tissue. Normally the excess amount of TGF-β enhances the production of collagen and fibronectin by fibroblasts. In the present study the formation of fibroblasts are also confirmed in the heart tissue (vide in chapter 3). At the same time the enhanced level of TGF-β inhibits the degradation of extra cellular matrix by stimulating the synthesis of protease inhibitors and decreasing the production of proteases, thereby favoring the accumulation of matrix proteins. In the heart, the TGF-β isoforms have been shown to be expressed at high levels during mercury intoxication. TGF-β is responsible for generated cytokine with an important function for healing and tissue fibrosis which is occurred during the mercury intoxication.
In normal animals, the heart tissue consisting solely of muscle cells because it is homogenous organ. However, number of research work suggests that the heart is partly made up of connective tissue cells (fibroblasts). These connective tissue cells help to produce an extracellular matrix which allows for fibrosis within the myocardium. The function of this fibrosis is still not completely known. Maintenance of myocardial architecture is mainly depending upon the networks of elastic tissue with in the heart. Collagen networks are also thought to be involved in the transmission of force generated in heart muscle tissue. Unfortunately, excessive deposition of fibrotic tissue in the heart results in cardiac pathology. Raised levels of collagen within the myocardium cause reduced heart function and also promoting chamber stiffness. This process leads to impaired myocyte relengthening during relaxation. In fact, studies indicate that fibrosis may contribute greatly to cardiac dysfunction. Hanna et al. (2004) noted that TGF-β1 levels were increased in the atria after the development of congestive heart failure in dogs. The most important finding in the present experimental study was mercuric chloride induced by cardiac fibrosis through enhancing the TGF-β1 signalling pathway. TGF-β1 has been shown to regulate the expression of procollagen genes and promote the synthesis of extracellular matrix components, thereby contributing to myocardial fibrosis (Khan and Sheppard, 2006). The appearance of fibrosis in particular organ leads to formation of pathology and dysfunction. In most of the studies are explained that the formation of common fibrotic process caused different type of cardiac dysfunction and its damages. Within the heart, this fibrosis is thought to be partially mediated by transforming growth factor-β1 (TGF-β1), a potent stimulator of collagen-producing cardiac fibroblasts. Previously, TGF-β1 had been implicated solely as a modulator of the myocardial
remodelling seen after infarction. However, recent studies indicate that dilated, ischaemic and hypertrophic cardio-myopathies are all associated with raised levels of TGF-β1. The reactive oxygen species (ROS) generations are also capable of inducing TGF-β1 activation. TGF-β1 expression is increased in the mercury intoxicated heart tissue. Oxidative stress is an important contributor to pathological remodeling, in the failing heart and plays critical role in stress responses myocardial remodeling (Kinugawa et al., 2000). In the present experimental study, treated rats with mercuric chloride express high levels of cardiac TGF-β1. These findings suggest the possible involvement of TGF-β1 genes in the regulation of cardiotoxicity process.

During the recovery treatment, Ferulic acid and methanolic fractions of *Terminalis arjuna* seed extract on mercury intoxicated rats showed decreased in the level of mRNA in TGF-β1 gene expression to near normal level. The result suggested that TGF-β1 secretion is increased following chronic administration of mercuric chloride could promote the heart injury. Further, also it suggests that expression of this cytokine may be regulated differentially during distinct phases of cardiac injury in rat. In addition, the finding is suggested that heavy metals, particularly mercury, interfere with the secretion of TGF-β1 offers a new mechanism to be considered in mercuric chloride induced cardiotoxicity. Taken together, these studies suggest that mercuric chloride exposure is associated with a proinflammatory process. The present experimental study was suggested that treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract have a preventive and protective effect of cardiotoxicity in mercuric chloride intoxicated rats. This result suggests that modulation of collagen
production and/or degradation by TGF-β1 may contribute to changes in myocardial structure and function. Similar type of result was observed by Al-Shabanah et al. (2012) in doxorubicin (DOX) induced cardiotoxicity in rats when treated with desferrioxamine (DFX) for post treatment. They are suggested that an administration of DFX prior to DOX resulted in a complete reversal of DOX-induced alteration in cardiac enzymes and gene expression to normal levels. Thus far, medical therapy targeting TGF-β1 has shown promise in a multitude of heart diseases. These therapies provide great hope, not only for treatment of symptoms but also for prevention of cardiac pathology as well. The protective effect of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract were clearly reflected in returning the cardiac enzymes and isoenzymes after the post treatment to their normal levels.