5. PROTECTIVE EFFECT OF FERULIC ACID AND METHANOLIC FRACTIONS OF *Terminalia arjuna* SEED EXTRACT ON SERUM ENZYMES MARKERS (ALT, AST, ALP) ACTIVITIES OF MERCURY INTOXICATED RATS

5.1 Introduction

Increasing pollution of heavy metal compounds is becoming a significant environmental problem (Margarat *et al.*, 2001; Kavitha and Jagadeesan, 2004; Jagadeesan, 2004; Jagadeesan and S. Sankarsami Pillai, 2007; Vanithasri and Jagadeesan; 2013, Bharathi and Jagadeesan, 2014). Among the heavy metals mercury and its compounds were formerly used in manufacturing of various products such as insecticides, batteries, as an antiseptic, disinfectant, preservative, in metallurgy, and as a photographic fixative (Jagadeesan and Sankarsami Pillai, 2007; Vanithasri and Jagadeesan, 2013). From the mercurial compounds one of the most toxic forms is mercuric chloride. During the formation of organomercury complexes in the target organs the accumulated mercuric chloride mainly bound with protein substances (Jagadeesan and Sankarsami pillai, 2007; Vanithasri and Jagadeesan, 2013; Mohamed, 2010). The absorbed mercury and its compounds mainly accumulated in the kidney but the metabolic process carried out in the liver resulting in severe cell damages occurred in target organs like heart tissue (Jagadeesan and Sankarsami Pillai, 2007).

Exposure of Mercuric chloride is promoting adverse health effects including cardiotoxicity, hepatic toxicity and renal toxicity in animals (Vanithasri and Jagadeesan, 2013; Bharathi and Jagadeesan, 2014; Vijayakumar *et al.*, 2014). Accumulation of mercury toxicity promotes the reactive oxygen species (ROS)
formation (Miller et al., 1991; Hussain, 1999; Kavitha and Jagadeesan, 2006; Jagadeesan and Sankarsami pillai, 2007; Bharathi et al., 2012; Vanithasri and Jagadeesan, 2013). An enhanced level of ROS subsequently promote the lipid peroxides and hydroxyl radical which may cause the cell membrane damage and thus destroy the cell of targeted organs in the intoxicated animals (Hussain, 1999; Jagadeesan and Sankarsami pillai, 2007). Mercuric chloride may induce heart cell membrane structural alterations through the ROS effect. In most cases the final major toxic form of mercuric chloride found in the affected blood and cardiac tissue (Kavitha and Jagadeesan, 2006; Bharathi et al., 2012; Vijayakumar et al., 2014). Mercury and its compounds also induced organs damages and it was detected by a significant increase in serum ALT, AST and ALP activities (Jagadeesan and Sankarsami pillai, 2007; Mohamed, 2010; Oda and El-Ashmawy, 2012).

One of the important enzyme lactate dehydrogenase (LDH) is found in all cells of normal animals (Thippeswamy et al., 2009; Vijayakumar et al., 2014). During the physical activity of an animal, this enzyme is playing a vital role for metabolizing lactate content (Senthil et al., 2007; Jahan et al., 2012). LDH is responsible for increasing the myocardial function to act as a diagnostic marker of heart tissue (Gnanapragasam et al., 2004). It has been reported that these enzymes are released from the heart into blood stream, thus increasing their concentration in serum level (Sathish et al., 2003; Deepa and Varalakshmi, 2003). This isoenzyme (LDH) is found abundantly in the cardiac tissue, liver, kidneys and striated muscle. When these organs are diseased or injured the LDH will accumulate in the body.
Another important enzyme creatine phosphokinase (CPK) is found mainly in cardiac muscle cells. It plays a vital role for supplying the energy during the transfer of phosphate substance (Vijayakumar et al., 2014). Determination of the CPK activity in the serum is mainly used to recognize the level of damages occurred in cardiac muscle (Gutenbrunner and Gutenbrunner, 2000; Vijayakumar, 2014). The overall activity of CPK comprises the activities of the isoenzymes creatinine kinase which are responsible for various heart functions (Thomas 1992). So determination of the CPK activity is useful in diagnosing cardiovascular disease. Accumulation of cholesterol content in the blood is also associated with an increased risk of heart attack (Jain et al., 2007).

With this point of view, the aim of the present experimental work was to find out the possibility to use the herbal medication such as methanolic fractions of “Terminalia arjuna” seed extract and “Ferulic acid” for protection of heart, liver and kidney from cellular damage induced by mercury chloride (HgCl₂) in the rats. These cellular damages are monitored through the certain serum bio-marker enzymes activities.

5.2 Observation

5.2.1 Alanine transaminase (ALT) enzyme activities

In the normal untreated control rat, Rattus norvegicus, the level of serum Alanine transaminase (ALT) activity present in the serum were 33.50 ± 2.89 IU/L. At sub-lethal dose of HgCl₂ treatment for 45 days, the level of ALT activity was drastically increased in serum respectively. During the post-treatment of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract on mercury
intoxicated rat serum were showed a decreased level of ALT activity respective treated groups (Table 6 and Fig. 14).

5.2.1.1 Mercuric chloride (HgCl$_2$) treated groups

During the mercury intoxication the serum shows a significant increase in the level of alanine transaminase (ALT) activities upto 73.50 ± 2.66 IU/L, when compared with untreated control. The percentage change over the control was +119.40 (Table 6 and Fig. 14).

5.2.1.2 Mercuric chloride followed by Ferulic acid treated groups

During the Ferulic acid treatment on mercury intoxicated rats, the increased level of alanine transaminase (ALT) enzymes activities was decreased to reach near normal level 45.35 ± 2.03 IU/L. The percentage change over the mercury treatment was -38.29 (Table 6 and Fig. 14).

5.2.1.3 Mercuric chloride followed by Methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the methanolic fractions of *Terminalia arjuna* seed extract treatment on mercury intoxicated rats, the increased level of serum alanine transaminase (ALT) enzymes activities was decreased to reach near normal level 40.73 ± 2.27 IU/L. The percentage change over the mercury treatment was -44.58 (Table 6 and Fig. 14).

5.2.1.4 Ferulic acid alone treated groups

The level of alanine transaminase (ALT) enzymes activities in serum was decreased in Ferulic acid alone treatment 31.50 ± 2.05 IU/L. The percentage change over the control was -5.97 (Table 6 and Fig. 14).
5.2.1.5 Methanolic fractions of *Terminalia arjuna* seed extract alone treated groups

The level of alanine transaminase (ALT) enzymes activities in serum was decreased in methanolic fractions of *Terminalia arjuna* alone treatment 30.72±2.31 IU/L. The percentage change over the control was -8.21 (Table 6 and Fig.14).

5.2.2 Aspartate amino transaminase (AST) enzyme activities

In the normal untreated control rat, *Rattus norvegicus*, the level of serum aspartate amino transaminase (AST) activity present in the serum were 43.11 ± 2.80 IU/L. At sub-lethal dose of HgCl$_2$ treatment for 45 days, the level of AST activity was drastically increased in serum respectively. During the post-treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat serum showed a decreased level of AST activity respective treated groups.

5.2.1.1 Mercuric chloride (HgCl$_2$) treated groups

During the mercury intoxication the serum shows a significant increase in the level of serum aspartate amino transaminase (AST) activities upto 138.25 ± 1.84 IU/L, when compared with untreated control. The percentage change over the control was +220.69 (Table 6 and Fig. 14).

5.2.1.2 Mercuric chloride followed by Ferulic acid treated groups

During the Ferulic acid treatment on mercury intoxicated rats, the increased level of serum aspartate amino transaminase (AST) activities was decreased to reach near normal level 72.29 ± 2.24 IU/L. The percentage change over the mercury treatment was -48.71 (Table 6 and Fig. 14).
5.2.1.3 Mercuric chloride followed by Methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the methanolic fractions of *Terminalia arjuna* seed extract treatment on mercury intoxicated rats, the increased level of serum aspartate amino transaminase (AST) activities was decreased to reach near normal level 64.55 ± 2.34 IU/L. The percentage change over the mercury treatment was -53.30 (Table 6 and Fig. 14).

5.2.1.4 Ferulic acid alone treated groups

The level of aspartate amino transaminase (AST) activities in serum was decreased in Ferulic acid alone treatment 42.50 ± 2.36 IU/L. The percentage change over the control was -1.41 (Table 6 and Fig. 14).

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

The level of aspartate amino transaminase (AST) activities in serum was decreased in methanolic fractions of *Terminalia arjuna* alone treatment 41.62 ± 2.22 IU/L. The percentage change over the control was -3.45 (Table 6 and Fig. 14).

5.2.3 Alkaline phasphatase (ALP) enzyme activities

In the normal untreated control rat, *Rattus norvegicus*, the level of serum alkaline phasphatase (ALP) enzymes activity present in the serum were 251.44 ± 2.95 IU/L. At sub-lethal dose of HgCl$_2$ treatment for 45 days, the level of ALP activity was drastically increased in serum respectively. During the post-treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat serum were showed a decreased level of ALP activity respective treated groups.
5.2.1.1 Mercuric chloride (HgCl₂) treated groups

During the mercury intoxication the serum shows a significant increase in the level of serum alkaline phosphatase (ALP) activity up to 310.39 ± 2.29 IU/L, when compared with untreated control. The percentage change over the control was +23.44 (Table 6 and Fig. 14).

5.2.1.2 Mercuric chloride followed by Ferulic acid treated groups

During the Ferulic acid treatment on mercury intoxicated rats, the increased level of serum alkaline phosphatase (ALP) activity was decreased to reach near normal level 271.69 ± 2.14 IU/L. The percentage change over the mercury treatment was -12.46 (Table 6 and Fig. 14).

5.2.1.3 Mercuric chloride followed by Methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the methanolic fractions of *Terminalia arjuna* seed extract treatment on mercury intoxicated rats, the increased level of serum alkaline phosphatase (ALP) activity was decreased to reach near normal level 276.15 ± 2.64 IU/L. The percentage change over the mercury treatment was -11.03 (Table 6 and Fig. 14).

5.2.1.4 Ferulic acid alone treated groups

The level of alkaline phosphatase (ALP) activity in serum was decreased in Ferulic acid alone treatment 250.95 ± 2.05 IU/L. The percentage change over the control was -0.19 (Table 6 and Fig. 14).
5.2.1.5 Methanolic fractions of *Terminalia arjuna* seed extract alone treated groups

The level of alkaline phosphatase (ALP) activity in serum was decreased in methanolic fractions of *Terminalia arjuna* alone treatment 248.91 ± 2.75 IU/L. The percentage change over the control was -1.00 (Table 6 and Fig. 14).

5.2.4 Lactate dehydrogenase (LDH) enzyme activities

In the normal untreated control rat, *Rattus norvegicus*, the level of Lactate dehydrogenase (LDH) enzymes activity present in the serum were 56.27 ± 3.38 IU/L. At sub-lethal dose of HgCl$_2$ treatment for 45 days, the level of LDH activity was drastically increased in serum respectively. During the post-treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat serum were showed a decreased level of LDH activity respective treated groups. (Table 7 and Fig. 15).

5.2.4.1 Mercuric chloride (HgCl$_2$) treated groups

During the mercury intoxication the serum shows a significant increase in the level of serum Lactate dehydrogenase (LDH) activity upto 183.33 ± 3.78 IU/L, when compared with untreated control. The percentage change over the control was +225.80 (Table 7 and Fig.15).

5.2.4.2 Mercuric chloride followed by Ferulic acid treated groups

During the Ferulic acid treatment on mercury intoxicated rats, the increased level of serum Lactate dehydrogenase (LDH) activity was decreased to reach near normal level 74.37 ± 2.31 IU/L. The percentage change over the mercury treatment was -59.43 (Table 7 and Fig.15).
5.2.4.3 Mercuric chloride followed by Methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the methanolic fractions of *Terminalia arjuna* seed extract treatment on mercury intoxicated rats, the increased level of serum Lactate dehydrogenase (LDH) activity was decreased to reach near normal level 67.15±3.20 IU/L. The percentage change over the mercury treatment was -63.36 (Table 7 and Fig.17).

5.2.4.4 Ferulic acid alone treated groups

The level of Lactate dehydrogenase (LDH) activity in serum was decreased in Ferulic acid alone treatment 54.74 ± 2.43 IU/L. The percentage change over the control was -2.71 (Table 7 and Fig. 15).

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

The level of Lactate dehydrogenase (LDH) activity in serum was decreased in methanolic fractions of *Terminalia arjuna* alone treatment 52.46 ± 3.08 IU/L. The percentage change over the control was -6.78 (Table 7 and Fig.15).

5.2.5 Creatine phosphokinase (CPK) enzyme activities

In the normal untreated control rat, *Rattus norvegicus*, the level of Creatine phosphokinase (CPK) enzymes activity present in the serum were 129.40±2.63 IU/L. At sub-lethal dose of HgCl₂ treatment for 45 days, the level of CPK activity was drastically increased in serum respectively. During the post-treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat serum were showed a decreased level of CPK activity respective treated groups. (Table 7 and Fig. 15).
5.2.5.1 Mercuric chloride (HgCl\(_2\)) treated groups

During the mercury intoxication the serum shows a significant increase in the level of serum Creatine phosphokinase (CPK) activity upto 203.22 ± 2.96 IU/L, when compared with untreated control. The percentage change over the control was +57.04 (Table 7 and Fig. 15).

5.2.5.2 Mercuric chloride followed by Ferulic acid treated groups

During the Ferulic acid treatment on mercury intoxicated rats, the increased level of serum Creatine phosphokinase (CPK) activity was decreased to reach near normal level 143.82 ± 2.18 IU/L. The percentage change over the mercury treatment was -29.22 (Table 7 and Fig. 15).

5.2.5.3 Mercuric chloride followed by Methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the methanolic fractions of *Terminalia arjuna* seed extract treatment on mercury intoxicated rats, the increased level of serum Creatine phosphokinase (CPK) activity was decreased to reach near normal level 138.00 ± 2.20 IU/L. The percentage change over the mercury treatment was -46.25 (Table 7 and Fig. 15).

5.2.5.4 Ferulic acid alone treated groups

The level of Creatine phosphokinase (CPK) activity in serum was decreased in Ferulic acid alone treatment 127.93 ± 1.92 IU/L. The percentage change over the control was -1.13 (Table 7 and Fig. 15).
5.2.5.5 Methanolic fractions of *Terminalia arjuna* seed extract alone treated groups

The level of Creatine phosphokinase (CPK) activity in serum was decreased in methanolic fractions of *Terminalia arjuna* alone treatment 125.62 ± 2.10 IU/L. The percentage change over the control was -2.92 (Table 7 and Fig.15).

5.2.6 Total cholesterol (TC) level

In the normal untreated control rat, *Rattus norvegicus*, the level of Total cholesterol (TC) content present in the serum were 75.93 ± 2.80 IU/L. At sub-lethal dose of HgCl$_2$ treatment for 45 days, the level of Total cholesterol (TC) content was drastically increased in serum respectively. During the post-treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat serum showed a decreased level of Total cholesterol (TC) content respective treated groups.

5.2.6.1 Mercuric chloride (HgCl$_2$) treated groups

During the mercury intoxication the serum shows a significant increase in the level of serum Total cholesterol (TC) content upto 141.00 ± 2.53 IU/L, when compared with untreated control. The percentage change over the control was +85.69 (Table 8 and Fig. 16).

5.2.6.2 Mercuric chloride followed by Ferulic acid treated groups

During the Ferulic acid treatment on mercury intoxicated rats, the increased level of serum Total cholesterol (TC) content was decreased to reach near normal level 82.84 ± 2.87 IU/L. The percentage change over the mercury treatment was -41.24 (Table 8 and Fig. 16).
5.2.6.3 Mercuric chloride followed by Methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the methanolic fractions of *Terminalia arjuna* seed extract treatment on mercury intoxicated rats, the increased level of serum Total cholesterol (TC) content was decreased to reach near normal level 78.31 ± 1.91 IU/L. The percentage changes over the mercury treatment was -44.46 (Table 8 and Fig. 16).

5.2.6.4 Ferulic acid alone treated groups

The level of Total cholesterol (TC) content in serum was decreased in Ferulic acid alone treatment 74.25 ± 2.40 IU/L. The percentage change over the control was -2.21 (Table 8 and Fig. 16).

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

The level of Total cholesterol (TC) content in serum was decreased in methanolic fractions of *Terminalia arjuna* alone treatment 72.51 ± 2.13 IU/L. The percentage change over the control was -4.50 (Table 8 and Fig. 16).
5.3 Discussion

Heavy metal exposure has been linked to increased incidence of cardiovascular diseases (Vijayakumar et al., 2014). Mercury is having one of the highly metal toxicity in board environmental and industrial pollution which induced various diseases in human and animal (Jagadeesan, 2004). In the present experimental study, for the first time, that 45 day treatment with a sub-lethal dose of mercuric chloride induce not only organ damages and also promote cardiac dysfunction due to increased free radical production and simultaneously decreased antioxidant properties. This is evidenced by the following effects of treatment. Hence, toxicity of mercury exposure is in part a function of increased oxidative stress (Bharathi et al, 2014). The increase in oxidative stress is possibly from the depletion of thiol compounds, inhibition of antioxidant enzymes, or both (Durak, et al., 2010; Bashandy et al., 2011) leading to cell injury damage to lipid peroxidation (Oda and El-Ashmawy, 2012; Sankarsami pillai et al., 2010). Free radical production and oxidative stress is one of the mechanisms of tissue damage in heavy-metal toxicity in animals. In the present experimental study, the level of LPO content increased enormously in heart, liver and kidney tissues of rat when treated with sub-lethal dose of mercuric chloride for 45 days. During mercury intoxication mercury toxicity promotes the hydroxyl free radicals in the target tissues. Mercury and its compounds cause’s cell membrane damages through the formation of lipid peroxidation (via ROS production) which leads to the imbalance between synthesis and degradation of enzyme protein (vide in chapter 4).
occurrences of target organs cells damage can be evaluated by bioenzymological analysis of the marker enzymes serum tests such as serum amino transferases and other enzymes. Serum amino transferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate the concentration of enzymes that have leaked into the blood circulation. When the rats were treated with HgCl₂, enhanced levels of biomarkers were observed in the serum. This finding could be a consequence of a reduction in the number of viable myocytes due to enhanced cell death in heart as these animals showed the enhanced level of serum marker enzymes (Vijayakumar et al., 2014). The excess production of ROS by mercury may be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore (Kavitha and Jagadeesan, 2003). These results may be due to target organ cells necrosis which causes increase in the permeability of cell membrane resulting in the release of these enzymes in the blood stream. In the present experimental study, during mercury intoxication produced organ damages (heart, liver and kidney damages) as evidenced by substantial increase in the leakage of AST, ALT and ALP into the circulation due to target organs cells necrosis or alteration in permeability of the cell membrane.

These antioxygenic enzymes (AST, ALT and ALP) are playing a vital role to scavenge the toxicants from the cell and also maintain the internal environment of the organs from the adverse effect of mercury toxicity even in lower concentration. An increase in the oxidative stress may be due to a decrease in the antioxidant defenses or due to an increase in the processes that produce oxidants.
might alter the enzyme profiles (Hussain et al., 1999; Sener et al., 2003). In the present experimental study, HgCl₂ initiate lipid peroxidation by generating free radicals and thereby interfering with the antioxidant system of the cell (vide in chapter 4). The accumulation of mercury toxicity might alter the membrane integrity via the formation of ROS and the perturbation of antioxidant defense mechanisms (Clarkson and Magos, 2006). In this respect, biomarker enzymes activity alterations may result from mercuric chloride effect primarily on heart, liver and renal tissues.

In the present experimental study, the level of AST and ALT activities are drastically increased in the serum of mercury intoxicated rat. Normally, alterations in the level of AST and ALT activities are reported in hepatic damages occurred in various animals (Margarat et al., 2001; Jagadeesan and Sankarsami Pillai, 2007). Because most of the authors are considered the bio-markers for liver functions are AST, ALT and ALP enzyme activities (Martin et al., 1981; Guha Mazumder, 2008). The principle action of AST is responsible for transferring amino group from aspartate to α-β glutaric acid forming glutamate and oxaloacetate (Sabeena et al, 2004; Rajadurai and Prince, 2007; Zhou et al., 2006; Vijayakumar et al., 2014). The increased level of AST in the blood stream is responsible for all types of target organ disease. Its peak concentration and ratio to other enzymes reflect the type of organ damage (Tiwari and Srivastava, 2001). The principle action of ALT activity is also responsible for transferring an amino group from alanine to α- ketoglutaric acid forming glutamate and pyruvate. It is also known that AST is very specific enzyme for heart, hepatic and kidney tissues. This enzyme is more sensitive to
target organ damages. Tiwari and Srivastava (2001) have been observed that the AST level rises faster and higher in most types of target cellular damage. The present experimental study shows significant increase in the level of AST and ALT in the serum of rat treated with sub lethal dose of mercuric chloride for 45 days. This result indicates that the increase in AST and ALT in serum may be due to cellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminase in the blood stream. Similar results were also observed by Sankarsami Pillai and Jagadeesan (2009) in mercury intoxicated rats. They are reported that the increased level of serum AST and ALT activities are mainly due to mercury toxicity in chronic hepatic disease indicating toxic liver damage. The increased level of serum AST and ALT activity in rats exposed to mercuric chloride may reveal possible leakage of these enzymes across damaged plasma membranes and or the increased synthesis of this enzyme. Meanwhile, the elevation of ALP activities are also correlates with heavy metal exposure level of animals. The increased serum ALP has been explained by pathological processes such as cardiac damages, liver impairment and kidney dysfunction (Bogin et al., 1994; Atroshi et al., 2000; Bharathi et al., 2014; Vijayakumar et al., 2014). Because, ALP is a brush border enzyme which play a vital role to split the various phosphate esters at an alkaline condition and mediates membrane transport (Smith et al., 1983). According to Flora et al. (1994), ALP is a membrane bound enzyme and its inactivation leads to membrane damage of cells. These enzymes are indicative of various aspects of metabolism and they have been used to evaluate the physiological, biochemical and metabolic defects in the heart, liver and kidney tissues. The significant increase in the activity of alkaline phosphatases may be
attributed to the destruction of all membranes and lysosomes which in turn might cause tissue damage (Saxena and Sarin, 1980; Ramalingam et al., 1999). Similar observations were made by various authors (Teotia and Teotia, 1991; Margarat et al., 2001; Kavitha and Jagadeesan, 2006; Sankarsami Pillai and Jagadeesan, 2007; Vanithasri and Jagadeesan, 2013; Bharathi and Jagadeesan, 2014).

In normal animal, the heart tissue cells contain many cardiac enzymes like aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and etc. During mercury intoxication, the oxygen demand of the heart increases with increase in inotropic effect in the heart. Due to this effect the heart cells are damaged with increased muscle contractility. Increasing the muscle contractility of the heart (vide in Chapter 3) results in increased membrane permeability which can promote the cardiac marker enzymes to leak out into the blood stream (Wang et al., 2009). These enzymes lack specificity may produce particular organ toxicity in mercury intoxicated rat. Because they are also present in organs other than the heart, e.g. liver, kidney and red blood cells, so that increased serum activities of these enzymes may reflect cardiac necrosis or red blood cell lysis in addition to cardiac toxicity necrosis.

In the present experimental study, the level of cholesterol content was drastically increased in the serum of mercury intoxicated rat. The result suggested that a high level of cholesterol in the blood serum is associated with an increased risk of heart attack in animals. Because enhanced level of cholesterol content is deposited in arterial walls which is the major component of the formation of plaques (Jain et al., 2007). During this period, the enhanced level of total
cholesterol content cannot dissolve in the blood. But it is combined with specific
types of special proteins to form lipoproteins (Radhika et al., 2012). Due to the
mercury toxicity, the enhanced level of cholesterol contents was carried by the
cholesterol carriers in blood circulation can deposit cholesterol in arterial wall to
promote the atherosclerotic plaque formation process (Vijayakumar et al., 2014).
For these reasons high levels of cholesterol content appeared in the blood serum
have a positive correlation with heart disease (Adebayo et al., 2007). Similar type
of result was also observed by Pamidiboina et al. (2010) in isoprenaline induced
hyperlipidema in rats. They are reported that an enormous increase of lipid
peroxidation through free radicals formation resulting in irreversible cellular
damages promoted to heart and aorta in isoprenaline treated rat. Recently Radhika
et al. (2012) have also observed the similar type of results in rat when treated with
isoproterenal. They are suggested that administration of isoproterenal causes an
enhanced level of lipid profiles in the blood circulation and also in heart tissues.
Enhanced level of cholesterol content in blood circulation can promote the
deposition of lipid profile in cardiac tissue are well associated with cardiovascular
damage. The present experimental study also confirms these results. Accumulation
of cholesterol plays a vital role in promotion of cardiovascular disease, not only by
way of development of atherosclerosis, but also by modifying the composition,
structure and stability of cellular membranes. High levels of circulating cholesterol
and its accumulation in heart tissue are well associated with cardiovascular
damage.
In normal animal the lactate dehydrogenase enzyme is found in blood and tissue (Vijayakumar et al., 2014). It plays a vital role of cellular respiratory process by which glucose from food is converted into usable energy for cells (Rajkumar and Ansar Kamran, 2013). The low level of LDH enzyme is normally present in the blood of an animal and at the same time high level of LDH enzyme abundantly present in tissue cells (Jagadeesan and Bharathi, 2014). LDH has several molecular forms called isoenzymes. Some LDH isoenzymes are present in certain tissues to a greater extent than in others (Bishop et al., 1972). When one of these particular tissues is damaged, an isoenzyme of LDH is released into the blood. In that case, determination of the pattern of LDH isoenzymes in serum may help to identify which tissue has been damaged. However, when tissues are damaged by injure, they release more LHD enzyme into the blood cells. In the present experimental study, the level of LDH activity was increased enormously in the blood of mercury intoxicated rat. The result suggested that the increased LDH levels in blood cells indicate heart, liver and kidney cell damages.

Mercuric chloride is well known cardiotoxicity agent due to its ability it will destruct heart tissue particularly myocardial cells. As a result of this, cytosolic enzymes such as Lactate Dehydrogenase (LDH) transaminase activity and Creatine Phosphokinase (CPK) were present in normal level in untreated animal blood. Determination of these contents are very helpful tool for diagnosing heart tissue damage. This finding could be a consequence of a reduction in the number of viable myocytes due to enhanced cell death in heart as these animals showed the
enhanced level of serum marker enzymes (Vijayakumar et al., 2014). Similar types of results were also observed by Khalifa and Elmazny, (2013) in CCl₄ intoxicated rats. Most of the study demonstrated that due to the mercury toxicity, the formation of free radicals initiate lipid peroxidation resulting in alteration of membrane integrity, fluidity and permeability in cardiac tissue (Mishra et al., 2011; Ramadoss et al., 2012). The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability.

In the present experimental study, due to the mercury intoxication, the oxygen demand of the heart increases with increase in isotropic effect in the heart, resulting in cardiac cell damages (vide in chapter 3). During the mercury intoxication the level of LPO content was drastically increased in heart tissues (vide in chapter 4) and simultaneously decreased level of antioxidant properties was also noticed. This result suggests that enhancement of free radicals cause cardiac cell damages. The cardiac cells are damaged with increased muscle contractility (vide in chapter 6), which results in increased membrane permeability allowing cardiac enzymes to leak out into the bloodstream (Wang et al., 2009). In the present experimental study, mercuric chloride treated rats showed significant elevation in the level of the diagnostic marker enzyme CPK. Moreover, an elevated level of this enzyme is an indicator of the severity of mercuric chloride induced heart tissue membrane necrosis. Assay of the activity of creatine phosphokinase in the serum is an important diagnosis, because of the marked abundance of this enzyme in heart tissue. When the heart cells are damaged, the cell membrane becomes permeable or may rupture, which results in the leakage of this creatine phosphokinase isoenzyme to the blood stream. This is the reason for the increased
activity of serum CPK in mercuric-induced cardio toxic rats. Similar type of result was also observed by Raju et al. (2008) in Wistar rat when treated with isoproterenol. They are suggested that the occurrence of heart failure subsequent to myocardial infarction may be associated with a decreased level of antioxidant properties as well as increased level of oxidative stress. Further, they are suggested that increased in the level of AST, LDH and creatinine kinase act as markers and are associated with higher incidence of heart attack. The present experimental results also prove this one.

The present experimental study clearly explained the number of pathophysiologic mechanisms in mercuric chloride induced heart, liver and kidney damages. The damaged organ tissues are possessing high amount of marker enzymes like CPK, LDH, ALT, AST and ALP. The level of these marker enzymes are served as sensitive index to assess the severity of organ damages particularly heart damages (Mastan et al., 2009; Gunjal et al., 2010). During the recovery period, administration of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract on mercury intoxicated rats shows the restoration of all marker enzymes in the serum in near normal level respectively. The result suggested that the administration of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract have also been found to prevent the myocardium infarction in mercuric chloride intoxicated rats. It may indicate the Ferulic acid and methanolic fractions of Terminalia arjuna seed extract in repairing the damaged organ tissues especially heart tissue and blood cells in cardiac system in mercury intoxicated animals. These finding might be rational to understand the beneficial effects of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract on cardiac
toxicity from mercuric chloride intoxicated rats. This reduction in enzyme levels could be due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes. It is therefore possible that antioxidant mechanisms might become activated in mercury exposed rats to protect cells against the increased oxidative stress (vide in chapter 4). These results may be attributed to the antioxidant activity of Ferulic acid and methanolic fractions of *Terminalia* seed extract and its constituents. Both Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract had a protective effect against HgCl₂ toxicity. Moreover, methanolic fractions of *Terminalia arjuna* seed extract was found to be more effective than Ferulic acid. This protection may be due to the free radical scavenger effect of these antioxidants and/or their enhancing effect on the antioxidant capacity of the body. Similar type of result was also observed by Dhana Rangesh Kumar *et al.* (2013) in Isoprenaline induced cardio toxicity in rats when treated with Garcinia indica fruit extract. They are suggested that treatment with fruit extract provide cardioprotection by inhibiting the formation of free radicals generated during oxidation of catecholamine thus inhibiting peroxidation of membrane lipids and preventing subsequent leakage of marker enzymes. Also, the fruit extract treatment improved the status of enzymatic antioxidants that further contributes to its overall cardio protective property. This is in agreement with our result.

In conclusion, the present experimental study suggested that sub-lethal dose of mercuric chloride treatment alters marker enzymes and induces heart dysfunction. This might be attributable, at least partly, to reductions in antioxidant bioavailability due to the increased ROS production in the target organs. Therefore,
mercury and its compounds could be considered an important risk factor for promoting cardiovascular disease. Administration of Ferulic acid and Methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rat shows the recovery from the adverse effect of mercury toxicity by the way of restoration of marker enzymes in near normal level.