3. PROTECTIVE EFFECT OF FERULIC ACID AND METHANOLIC FRACTIONS OF *Terminalia arjuna* SEED EXTRACT ON HISTOLOGY AND HISTOPATHOLOGICAL ALTERATION IN SELECTED TISSUES OF MERCURIC CHLORIDE INTOXICATED RATS

3.1 Introduction

Histological study appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the heart, liver and kidney tissues. Exposure to heavy metals may cause histological changes in the heart, liver and kidney tissues. It has been noted that heavy metals had a negative impact on all relevant parameters and caused histopathological changes in animals (Abdel-Warith *et al.*, 2011). Mercury (Hg), is a worldwide pollutant transported by air and water throughout the atmosphere, poses a great health risk to global health. The heavy metal mercuric chloride has been used for centuries both as a medicine and a poison and is currently used for many commercial purposes. Recently, attention has been refocused on this metal due to concern of environmental exposure. Hence, mercuric chloride is one of the heavy-metal, which is present in an inorganic mercury compound with ionic mercury. Many inorganic compounds of mercury have used in various products and agriculture medicines etc (Bharathi *et al.*, 2014).

The general structure of the circulatory system of the rat is almost identical to that of humans. Pulmonary circulation carries blood through the lungs for oxygenation and then back to the heart. The heart is the central organ of the circulatory system. It is unknown to what extent cardiovascular effects of mercury
are due to direct cardiac toxicity or indirect toxicity caused by effects on the neural control of cardiac function (Vijayakumar et al., 2014). Recently, more attention has been given to the toxic effects of mercury on the cardiovascular system (Wiggers et al., 2008). Nevertheless, the liver and kidney also accumulate high amounts of mercurial that may impair their regular functioning (Branco et al., 2011). Mercury can accumulate in the liver, resulting in severe hepatic damages. Previous studies have revealed that HgCl₂ caused histopathological and ultrastructural lesions in the liver evidenced by periportal fatty degeneration and cell necrosis (El-Shenawy and Hassan, 2008). Nephrotoxicity caused by to Hg²⁺ accumulation. The kidneys excrete waste products of metabolism and play an important role in maintaining the homeostasis by regulating the body water and solute balance (Bharathi et al., 2014). In addition to the excretory function, the kidney tissues also act as a harbor of mercury and its compounds has been show to accumulate in kidneys along with in other organs (Augusti et al., 2008). Mechanisms of tissue damage have been caused by mercury and its compounds are not completely clarified. But many studies confirm the promotion of oxidative stress as important factor in tissues damage (Bharathi et al., 2014). These oxidative stresses may cause the cell membrane damage and thus destroy the cell structure (Bashandy et al., 2011).

A specific concern associated with mercury exposure in humans is the need for effective therapy in dealing with intoxication. In this respect, chelating therapy is the most commonly used and seen as the least invasive (Oda and El-Ashmawy, 2012). Terminalia arjuna, a deciduous tree of Cobretaceae family, has been widely used in Indian System of Medicine in cardiac ailments (Vaidya, 1994). Numbers of experimental studies have reported beneficial effects of the plant parts powder of Terminalia arjuna in various organ diseases (Dwivedi and Jauhari, 1997;
Sumitra et al., 2001; Bharani et al., 2002). However, the mechanisms underlying its beneficial effects have not been properly evaluated. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous phenolic compound found in plant tissues and thus is a bioactive ingredient in many foods. Ferulic acid has been shown to exhibit antioxidant activity to improve blood fluidity. As an antioxidant, Ferulic acid plays a major role in the body’s defense against stress. Herbal plants are known to possess antioxidant activities, as they are rich in various antioxidant molecules. These plants are widely used in Ayurvedic medicine for the remedies for cardiac and other problems in humans and animals.

Histopathological changes serve as an important tool in the field of toxicological studies. If any biochemical or physiological deviations occur in the organ by the heavy metal, it may also alter the histological architecture of the tissue. It gives a clear picture of the effects imposed on the organ system by the metal (Basu et al., 2001). With this point of view, the aim of the present experimental work was to prove the possibility to use an herbal medication such as methanolic fractions of Terminalia arjuna seed extract and Ferulic acid for protection of the heart, kidney and liver from damage induced by mercury chloride (HgCl₂) in the rats through the histopathological observations.

3.2 Observation

3.2.1 Heart

3.2.1.1 Untreated Control

The major portion of the heart is made up of cardiac muscles. Normally cardiac muscle fibers are formed by individual muscle cells. One or two nuclei are present in the central portion of the muscle cells. A junction was noticed in-
between the two cardiac muscle cells. Physiologically, cardiac muscle is intrinsically rhythmic although it is regulated through nervous and hormonal mechanisms. The heart is made up of three layers, such as pericardium, myocardium, endocardium. The myocardium is the largest of the three layers, and contains cardiac muscle fibers, and loose endomysial connective tissue that contains lots of capillaries. This kind of muscle is found only in the heart. The layer of the heart consisting of cardiac muscle is called the myocardium. Its inner surface of the myocardium is lined with endocardium, and the outer surface with epicardium. Fig. H1 and H2 show normal untreated control cardiac muscle in longitudinal section. The striations can be seen along the length of the muscle fibres. The nuclei of the cardiac muscle cells lie in the middle of the cells. The myofibrils separate to bypass the nucleus, and there is often a perinuclear region in which no striations are seen. The untreated control group rat heart tissue shows the normal appearance of its histoarchitecture of myocardial membrane. The uniformed size and shape of the cardiac muscle fibers are noticed with prominent Fibroblast nuclei (Fig. H1 and H2) shows the regular arrangement of more number of cardiac cells in heart tissue.

3.2.1.2 Mercuric chloride (HgCl₂) treated groups

Histopathological examination of myocardial tissue obtained from normal control animals heart tissue shows clear integrity of myocardial membrane. At sub lethal dose of mercuric chloride, heart tissue shows completely damaged myocardium. The administration of mercuric chloride produced massive changes in the myocardium shows a varying degree of vacuolar changes in the cardiac muscle fibers mainly in the form of degeneration of myocardial tissue, vacuolization of the cardiomyocytes, cytoplasmic region infiltration of inflammatory cells and
myofibrillar changes. In mercuric chloride treated group, focal lesions in many sections consisting of molten staining and fragmentation of muscle fibres with confluent retrogressive lesions, hyaline necrosis, sequestering mucoid edema were also noticed in this sections (Fig. H 3 and H 4).

3.2.1.3 Mercuric chloride followed by Ferulic acid treated groups

During the recovery period, the mercury intoxicated animals were again treated with Ferulic acid for 45 days; the heart tissue shows the complete regenerated histoarchitecture. The restored size and shape of the cardiomyocytes, myocardial cells, fibroblast nuclei and myofibrils were noticed in all regions of the heart tissue. The numbers of vacuoles are also reduced and in some areas and it also disappeared (Fig. H5 and H6).

3.2.1.4 Mercuric chloride followed by methanolic fractions of *Terminalia arjuna* seed extract treated groups

During the recovery period, the mercury intoxicated animals were again treated with methanolic fractions of *Terminalia arjuna* seed extract for 45 days, the heart tissue shows the complete regenerated histoarchitecture. The restored size and shape of the cardiomyocytes, myocardial cells, fibroblast nuclei and myofibrils were noticed in all regions and disappearances of vacuoles are also noticed (Fig. H 7 and H 8).

3.2.1.5 Ferulic acid alone treated groups

The Ferulic acid alone treatment, the section of the heart tissue shows the restoration of normal histoarchitecture of cardiac myocytes, fibroblast and nuclei of myocardial membrane is evident (Fig. H 9 and H 10).
3.2.1.6 Methanolic fractions of *Terminalia arjuna* seed extract alone treated groups

The methanolic fractions of *Terminalia arjuna* seed extract alone treatment, the section of the heart tissue shows the restoration of normal histoarchitecture of cardiac myocytes, fibroblast and nuclei of myocardial membrane is evident (Fig. H 11 and H 12).

3.2.2. Liver

3.2.2.1 Untreated control

Fig. L1 shows the untreated control of the paraffin sectioned and eosin strained section of the liver tissue of rats. Fig. L1 shows the histoarchitecture of liver at low magnification. It is composed of parenchyma cells (hepatocytes) which are cylindrical in shape with a venous channel. The hepatocytes are uniformly arranged throughout the section. Hepatocytes are roundish, polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures known as hepatic cells cords. Under the higher magnification the kupffer cells are recognized by the shape of their nucleic. Fig. L2 shows that the hepatic cells cords are composed of one of two rows of hepatocytes. In between the rows, a tiny channel is formed which drains peripherally in lobule to bile ducts. The bile ducts are made up of simple cuboidal or columnar epithelial cells. The blood supply of the liver lobule is via the sinusoids, which from a sponge worle between the plats of hepatic cells. Central veins are centrally located in the lobules. In form the smallest radicals of the hepatic veins. Portal canals are surrounded by small amount of fibro connective tissue. The bile duct, portal vein and hepatic vein are collectively called portal triad (Fig. L2).
3.2.2.2 Mercuric chloride (HgCl$_2$) treated groups

At sub-lethal dose of mercuric chloride intoxication the rat liver tissue shows complete damage of its histoarchitecture, because mercuric toxicity has induced discrete pathological changes. The hepatocytes possess irregular size and shape. The distribution of the hepatocytes is not uniform in all regions (Fig. L3). Under the higher magnification the degeneration of cytoplasm was evident in most of the hepatocytes. In some area the clumping of hepatocytes is evident. In addition atrophic formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocytes cell wall and disposition of hepatic cords are also seen in all regions. Complete damage of bile ducts is also noticed. Due to mercuric chloride treatment, the size of the portal vein has also increased, and damaged columnar epithelium is evident (Fig. L4).

3.2.2.3 Mercuric chloride followed by Ferulic acid treated groups

Ferulic acid treatment on 45 days mercuric chloride intoxicated rat liver tissue shows a complete regeneration from the toxic effect of mercury. The number and size and shape of the hepatocytes are restored. The arrangements of hepatic cells are regular. Reappearance of bile duct is also evident for decreasing the mercury toxicity by Ferulic acid. Restoration of portal and central vein is also noticed. Restoration of hepatic cells cords and regenerated columnar or cuboidal epithelium lining in the bile duct is also evident for its regeneration promoted by Ferulic acid (Fig. L5 and L6).
3.2.2.4 Mercuric chloride followed by methanolic fractions of *Terminalia arjuna* seed extract treated groups

Methanolic fractions of *Terminalia arjuna* seed extract treatment on 45 days mercuric chloride intoxicated rat liver tissue shows a complete regeneration from the toxic effect of mercury. The number and size and shape of the hepatocytes are restored. The arrangements of hepatic cells are regular. Reappearance of bile duct is also evident for decreasing the mercury toxicity by methanolic fractions of *Terminalia arjuna* seed extract. Restoration of portal and central vein is also noticed. Restoration of hepatic cells cords and regenerated columnar or cuboidal epithelium lining in the bile duct is also evident for its regeneration promoted by methanolic fractions of *Terminalia arjuna* seed extract (Fig. L7 and L8).

3.2.2.5 Ferulic acid alone treated groups

Ferulic acid alone treatment groups rat liver shows the complete normal histoarchitecture as in untreated control animal. The regular size and shape of the hepatocytes are seen. The uniformed arrangement of hepatocytes and its porta-triad are evident (Fig. L9 and L10).

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

Methanolic fractions of *Terminalia arjuna* seed extract alone treatment group rat liver shows the incomplete histoarchitecture. The central vein is completely filled by blood mass. The size and shape and arrangement of hepatocytes are normal but in some areas densely granular hepatocytes are seen. Maintaining of hepatic cords is also noticed (Fig. L11 and L12).
3.2.3 Kidney

3.2.3.1 Untreated Control

The control group is eosin and heamatoxylin stained in kidney tissue shows the complete normal size and shaped in histoarchitecture of nephron, glomerules and renal cells. They are two types of uriniferous tubules one is nephron cells tubule and another one is collecting tubule. The renal corpuscles consist of glomerules and Bowman’s capsule. The functional unit of medullary layer contains a number of proximal and distal convoluted segment and tubules. In the distal convoluted tubules more convoluted cells are seen and they become a macula densa of the distal thick segment. The proximal convoluted tubules have a brush border and lumen (Fig. K1 and K2).

3.2.3.2 Mercuric chloride (HgCl$_2$) treated groups

At sub lethal dose of mercuric chloride intoxicated rats kidney tissue shows the pycnotic nucleus and thickening of the basement membranes. Vacuoles are also seen in all the regions. The size and shape of the nephron are reduced. Disappearance or damaged Bowman’s capsule is seeing in all regions. Damaged proximal and convoluted tubules are also noticed in Fig. K3 and K4 thinning of the macula densa layer is also seen. Brush broader has disappearance in proximal convoluted tubules.

3.2.3.3 Mercuric chloride followed by Ferulic acid treated groups

During the recovery periods, sections show the complete regenerated histoarchitecture of the kidney. The restored size and shape of the nephron are
noticed in all regions. Disappearance of vacuoles was also noticed in all regions. Regenerated proximal and distal tubules are seen in all regions. Reappearance of Bowman’s capsule and macula densa layer is also evident in regenerated tissue. Reappearance of brush border is also evident in proximal convoluted tubules (Fig. K5 and K6).

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

Methanolic fractions of *Terminalia arjuna* seed extract treated mercury intoxicated kidney tissue shows the incomplete histoarchitecture. The size and shape of the nephran are maintained. But in the Bowman’s capsule the bowman’s space is not visualized. In some are, brush border is not regenerated in the proximal tubules (Fig. K7 and K8).

3.2.3.5 Ferulic acid alone treated groups

Restoration of normal kidney histoarchitecture is evident in Ferulic acid alone treatment. The size and shape of bowman’s capsules are as in untreated rat. There is no difference in both the types of convoluted tubules (Fig. K9 and K10).

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

Restoration of normal kidney histoarchitecture is evident in methanolic fractions *Terminalia arjuna* seed extract alone treatment. The size and shape of bowman’s capsules are also maintained. There is no difference in both the types of convoluted tubules (Fig. K11 and K12).
3.3 Discussion

3.3.1 Heart

In all vertebrates the heart is a muscular organ which is present and responsible for pumping blood through the blood vessels by carry out the repeated rhythmic contractions (Heath et al., 1999). The muscular organ, heart, is composed of cardiac muscle (myocardium), an involuntary muscle tissue which is found only within this organ. The myocardium is the heart's muscular wall (Heath et al., 1999). It plays a vital role for contracts to pumping the blood out of the heart and then relaxes as the heart refills with returning blood. Outer surface of the myocardium is called the epicardium and its inner lining is the endocardium (Heath et al., 1999). In the present experimental study, histological examination of heart tissue of control rats showed normal myocardial fibers and muscle bundles with normal architecture (Fig. H1 and H2). Histological examination of heart tissue of mercury treated rats showed moderate hypertrophy of cardiomyocytes (Fig. H3 and H4). The mercuric treated in cardiac tissue is massive change in the myocardium showing a varying degree of vacuolar changes in the cardiac muscle fibers mainly in the form of degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells and myofibrillar loss. In mercuric chloride administered group, focal lesions in many areas showing the moltled staining and fragmentation of muscle fibres with confluent retrogressive lesions, hyaline necrosis, sequestering mucoid edema were observed. This type of histopathological examination of cardiac tissue revealed moderate to severe cardiomyopathy which includes myocyte degeneration, inflammatory cell infiltration, fibrous tissue proliferation, and necrotic foci in mercuric chloride treated heart tissues.
The histopathological examination of myocardial portions of the hematoxilin and eosin stained heart tissue revealed that the significant pathological lesions showed in mercury intoxicated rats. Hemorrhage, necrosis, mononuclear cell infiltration and fibrosis were noticed in the mercury intoxicated heart tissue due to its toxicity effect. Fig. H3 and H4 Shows the myocardial necrosis in the form of cogulative necrosis, hypereosinophilia of the myocyte, loss of striation and karyopyknosis of the nuclei in the myocardial muscles. In the present experimental study, the accumulation of lipid particles in the heart tissue reflected to the formation of oxidant free radicals generation. Due to the mercury toxicity the level of LPO content was drastically increased in heart tissue (vide in chapter 4) is supporting evidence for these histopathological changes which was attributed to accumulation of lipid (Badalzadeh et al., 2008). This is consistent with our pathological finding as mercuric chloride intake caused myocardial necrosis. This result suggested that the accumulation of mercury toxicity was increased in cardiac tissue of the experimental rats following treatment with mercury chloride. The high amount of mercury (not observed in the present experimental study) in the heart tissue might have brought about oxidative stress-induced damages. Rashid et al. (2013) had revealed that increased lipid peroxidation can negatively affect the membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors. In fact, reactive oxygen species have been implicated in the pathophysiology of a large number of diseases (Barp et al., 2002). Evidence from experimental as well as clinical studies suggests the role of oxidative stress in the pathogenesis of heart dysfunction (Piano, 2002). Furthermore, elevated ROS are implicated in the development of cardiac
hypertrophy, reperfusion injury, remodelling and heart failure (Sorescu and Griendling, 2002). The mechanisms by which ROS can damage cardiac muscle are multiple and certainly involve direct toxicity by inducing both necrosis and apoptosis (Chesley et al., 2000), impairing myocardial function. Similar type of histopathological observations also made by Manna et al. (2008) in arsenic intoxicated mice heart tissue. They have observed the cardiac segments of arsenic intoxicated mice reveals that arsenic treatment caused abnormal ultrastructural changes in the cardiac tissue. These type of abnormal changes occurred in cardiac tissue is mainly due to the over production of LPO content in heart tissue. The present experimental work also supporting this events (vide in chapter 4).

Now a day, attention has recently been given to toxic effects of mercuric chloride in the cardiovascular system (Evans and Weingarten, 1990; Salonen et al., 1995; 2000; Virtanen et al., 2005; Houston, 2007). Cardiac toxicity from mercuric chloride is associated with in vivo oxidative stress. Mercuric chloride exposure of animals or humans thus induces the generation of ROS with subsequent oxidative damage in several organs and systems (vide in chapter 4), as well as altering the antioxidant defence system in cells (Reus et al., 2003; Chen et al., 2005; Ognjanovic, 2008). Moreover, ROS accumulation induced by high concentrations of HgCl\(_2\) also results in cytotoxicity of endothelial cell monolayers (Wolf and Baynes, 2007). Despite the number of studies showing that mercuric chloride increases vascular resistance and induces oxidative stress, as well as risk for cardiac toxicity, the effect of in vivo chronic exposure to mercuric chloride on coronary vascular responses is unknown.
During the mercury intoxication, chronic and acute overproduction of ROS is integral part in the development of cardiovascular diseases in animals. Several animal models of oxidative stress have revealed that ROS plays a significant role in arteriosclerosis and other cardiovascular diseases (Harrison et al., 2003; Singh and Jialal, 2006). Accumulation of lipid peroxides and production of free radicals (vide in chapter 4) have been recognized as one of the possible mechanism for the cardiac damage induced by toxicants (Lemasters et al., 2004). Since HgCl$_2$ is a potent free radical inducer, oxidative stress may be one of the major pathways in mercuric chloride induced cardiac damages. Lams and Wexler (1979) had reported that administration of mercuric chloride not only promote the cardiac damages directly and also causes liver and kidney cirrhosis which interferes with lipid metabolism leading to heart failure, poor myocardial repair and atrial thrombosis.

During the recovery period, mercuric chloride followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract, the heart tissue shows the complete regeneration of its histoarchitecture. The myocardial cells such as cardiomyocytes and myofibrillar cells are seen in all regions of the completed regeneration of its histoarchitecturte. These cells size and shapes are restored. The cardiac membrane layers completely regenerated due to the antioxidant properties of by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract. There was also promotion in the number of cardiac myofibril in all the myocardial regions even complete regeneration of cardiac cells. Manna et al. (2007) have also observed the similar type of result in CCl$_4$ induced cardiotoxicity in rat. They suggested that CCl$_4$ treatment caused abnormal ultrastructural changes in the
cardiac tissue and that could be prevented by the administration of the active constituents of *Terminalia arjuna* a prior to toxin treatment. Hence, Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract confer protection to the myocardium through a free radical scavenger. Further, recovery treatment with the post administration of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract significantly attenuated the ultrastructural pathological changes in the cardiac tissue induced by mercuric chloride as evident from the light microscopic studies. As stated above, mercury and its compounds toxicity injury is considered to be a major clinical problem and occurs in many kinds of tissues, including cardiac and skeletal muscle (Harris *et al.*, 1996; Reiter and Tan, 2003). In addition to cardiac injury, ROS are thought to play an important role in many diseases (Benzie, 2000; Gate *et al.*, 1999; Stocker, 1999). The biochemical profile of the myocardium, like, depletion of SOD, catalase and glutathione (GSH) with increase LPO content provides strong evidence for oxidative stress occurring during cardiotoxicity induced in mercuric chloride intoxicated rat. The extent of myocardial lipid peroxidation (as estimated by TBARS) was found to be less in methanolic fractions *Terminalia arjuna* treated rat. The antioxidant enzymes like SOD and catalase activities were better preserved in recovery group (III) animals. These observations indicate a strong correlation between the preserved myocardial antioxidant status and the improved haemodynamic profile following cardiac tissue injury in Methanolic fractions of *Terminalia arjuna* seed extract treated rats. Protective role of the active constituents of Methanolic fractions of *Terminalia arjuna* seed extract was also supported from the histological studies. Combining all, it can be speculated that phytoconstituents of methanolic fractions of
Terminalia arjuna seed extract plays cardioprotective role against mercuric chloride induced myocardial oxidative stress probably via radical scavenging and enhancing antioxidant properties.

Ferulic acid is a natural antioxidant which is used to treat hypertensive diseases in China (Hou et al., 2004). Numbers of epidemiological studies have also provided ample evidence that a high concentration of Ferulic acid reduces and clear the risk of chronic diseases including cardiovascular diseases in animals (Pandey and Rizvi, 2009). Because, Ferulic acid is a phenolic compound that acts by scavenging free radicals and quenching lipid peroxidative chain which readily forms a resonance-stabilized phenoxy radical which accounts for its potent antioxidant activity. Similar types of results were also observed by Pamidiboina et al. (2010) in Isoprenaline induced cardiotoxicity in rats when again treated with polyherbal formulations. They have suggested that the Poly herbal (Antichol) posses cardio protective, antihyperglycemic activity in addition to antihyperlipidemic activity. The mechanism of antihyperlipidemic, antihyperglycemic, cardio protective activity may attribute to its antioxidant activity. Although there have been few studies on the tissue distribution of Ferulic acid after oral administration, Ferulic acid stays longer in blood than do other antioxidants and it would be useful for the prevention of oxidation damage in various tissues (Li et al., 2012). In the present experimental study, administration of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract protected myocardium from mercury induced myocardial functional and structural injury.
3.3.2 Liver

Liver is the first organ to face the toxicants in the body. It plays a vital role for the detoxification of toxicants including heavy metals through their portal system. The liver consists of hepatic cells which are carried out numerous functions. Carbohydrate, protein and lipid metabolisms are also involved in the liver organ. In addition to this the normal liver is engaged in excretory activities through their bile ducts. Liver carry out both exocrine and endocrine secretary functions. It has high concentrations of enzymes which are capable of bio transforming foreign chemicals. Because, the liver receives the absorbed substances form the digestive tract through the blood vessels directly. Along with the digested food substances the toxicants also entered in the liver. Most of the biotransformation of toxic substances is carried out in the liver organ due to their capacity to degrade the toxic substances with the help of antioxidant properties. If this capacity of the liver is overwhelmed by the various toxicants. The accumulated toxicants in the liver could promote the deleterious effect to causes cellular damages.

In the present experimental study, the disturbances of histological organization of hepatocytes were seen in the mercury intoxicated liver tissue. The irregular shape and size of the hepatocytes were noticed in mercury intoxicated liver (Fig. L3 and L4). The arrangement of hepatic cells not uniformly distributed. In most of the hepatic cells, cell border is not visible and damaged blood vessel and bile duct was noticed. Pycnotic hepatocytes are seen in (Fig. L3 and L4) the
central and portal veins are enlarged and also damaged in the border. Numerous vacuoles are appeared in the mercury intoxicated liver tissue is mainly due to the disintegration of hepatic cells in that area. It is one of the reasons for the presence of vacuoles in the present study. The present experimental study suggested that toxic responses occur relatively frequently in the liver compared with other organs, mainly because the liver is a predominant organ for the metabolism, and is also the first major organ to be exposed to ingested toxins. In the present experimental study, the accumulation of mercury toxicity causes reversible cellular damages due to its impact on the generation of free radicals (vide in chapter 4). These histological changes observed in the present study are similar to those observed by Jayakumar et al., (2008) in carbon tetra chloride exposed rat. The liver damages was mediated by free radicals as seen in the case of carbon tetra chloride exposed animals where the histopathological changes are due to free radicals formed from carbon tetra chloride.

In the present experimental study, the accumulation of mercury toxicity affects the liver tissue leading to necrosis of hepatocytes, vacuolization, karyolysis, cytoplasmoslysis, cirrhosis, nuclear enlargement, damaged cell boundaries and hemorrhage (Fig. L3 and L4). During the mercury exposure cell swelling was noticed in some area of the liver section. It indicate that the inflammation was occurred in the treated liver due to the enhancement of LPO content (vide in chapter) leads to cellular injury. Mercuric chloride is an inorganic compound mainly accumulated in kidney, liver, brain etc. Hence, the liver is not generally considered as a target organ in inorganic mercury toxicity. Chronic exposure to mercuric chloride leads to liver damage in rats as evidenced by peripheral lipid
accumulation and foci of hepatocyte necrosis. Similar types of results were also observed by Sankarsami Pillai (2006) in liver tissue of rats when treated with median lethal dose of mercuric chloride for 7 days. He stated that a fatty liver is a common response noted with a variety of liver toxicants and represents a potentially reversible injury of the hepatocytes. Additionally, he documented that the loss of hepatic cell integrity and homeostasis generally cause energy production failure and cell membrane rupture, which allow leakage of cell contents and enzymes. The observation of the mercury intoxicated liver section shows the damaged hepatic cells congestion of blood vessels and their rupture leading to internal haemorrhage has impeded the normal functions of mercury exposed rat.

Hepatotoxicity induced by mercuric chloride is a series of complex events with the help of reactive oxygen species. Over production of ROS play a vital role for causing the hepatotoxicity in animals. Most of the accumulated heavymetal toxicants promote the hepatotoxicity in animals. The liver injury via chemical hepatotoxictants led to increased lipid peroxidation and it is evident in the present study (vide in chapter 4). In the present experimental study, the liver section shows the increased size and shape of the Kupffer cells (Fig. L5 and L6). It indicates that the liver injury was mediated by kupffer cells. Two different type of mechanism was involved in which Kupffer cells mediate hepatotoxicity. One is a direct mechanism in which active oxygen species produced by kupffer cells to cause damages in the parenchymal cells. The other way is through the increased infiltration of inflammatory cells such as neutrophils, via increased production of cytokines released from activated kupffer cells (Yamano et al., 1998).
Acute liver diseases constitute a global concern, and medical treatments for these diseases are often difficult to manage and have limited efficacy. Therefore, there has been considerable interest in the role of complementary and alternative medicines for treatment of liver diseases (Seeff et al., 2001). Development of therapeutically effective agents from natural products may reduce the risk of toxicity when the drug is used clinically. Heavy metals particularly mercuric chloride is used as an experimental model of severe hepatic damage by generation of oxidative stress and activation of immune cells, which can lead to architectural and functional alteration (Hayden and Ghosh, 2008). As a result of hepatic injury, serum ALT and AST levels showed a marked increase after mercuric chloride treatment; however, these increases were attenuated by post treatments of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract respectively. Similar type of observations also made by Kim (2012) in CCl₄ treated liver when again treated with Ferulic acid. They have suggested that Ferulic acid protects from CCl₄ induced acute liver injury through reduction of oxidative pathways and inflammatory signaling pathways. In the present experimental study, histological observations of the liver sections strongly support the hepatoprotective effect of Ferulic acid and methanolic fractions of Terminalia arjuna seed extract respectively (Fig. L7 and L8). Mercuric chloride caused various histological changes to the liver, including cell necrosis, fatty metamorphosis in adjacent hepatocytes, ballooning degeneration, and infiltration of lymphocytes and Kupffer cells. These changes were significantly attenuated by Ferulic acid and methanolic fractions of Terminalia arjuna seed extract. These results indicate that Ferulic acid and methanolic fractions of Terminalia arjuna seed extract may have potential clinical application for treatment of liver diseases.
The present experimental study suggested that the administration of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract can be developed as a drug against mercuric chloride related disabilities in the opportunity. Moreover, it protects the animals from HgCl$_2$ induced hepatocytes and kupffer cells dysfunction and executes its modulating the histopathological alteration changes during free radical production. This experimental work suggested that treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract have a preventive and protective effect of the system from mercuric chloride intoxication.

### 3.3.4 Kidney

Kidneys are essential in the urinary system of animals. It also serves homeostatic functions such as the regulation of electrolytes, maintenance of acid base balance and regulation of blood pressure. The kidney plays a vital role in the body of the animals as a natural filter of the blood during the removal of unwanted water soluble wastes from the body. It also carries out the various processes such as degradation, detoxification and elimination of toxic substances. In the present experimental study, numerous changes are observed in the rat when treated with sub-lethal dose of mercuric chloride for 45 days. Some of the following changes degeneration of renal tubule and rupture of haemopoietic tissues, prominent changes in the proximal and distal tubules, vacuolization, mild necrosis and breakages of Bowman’s capsule are noticed in the kidney sections of mercury intoxicated rat (Fig. K1 and K2). Nephran is the basic structural unit of the kidney tissue which is composed of several types of cell layers. Due to this heterogeneity of the cell layers the toxicants produces cellular injury.
Mercury has been known to adversely affect the kidney tissues. In the present experimental study, mercuric chloride exerts potent nephrotoxic effects, including proximal tubule damages, development of nuclear inclusion bodies, interstitial fibrosis and tubular atrophy. Jagadeesan and Sankarsami pillai (2007) has been noticed similar type of damages in kidney organs of rat when treated with median lethal dose of mercuric chloride for 7 days. He has showed histologically damaged glomerulus, cloudy swelling to necrosis of the epithelium of the proximal convoluted tubules.

Chronic administration of mercuric chloride induces kidney damage in animals ultimately leading to death. These types of adverse effects induce renal dysfunction. In the present study the accumulation of mercury toxicity not only damage the glomerulus and also promoting the swelling and necrosis of the epithelium of the proximal convoluted tubules (Fig. K3 and K4) these type of major histopathological alterations occurred in the kidney tissue is mainly due to the accumulation of pollutants in an animal. The result suggested that degeneration in the tubular cells promote impairment in the reabsorption of electrolytes and infiltration process leading to imbalance in osmotic regulation of body fluids. These pathological changes are due to the preferential accumulation of pollutants that cause tubular degeneration leading to lymphocytic infiltration as a measure of resistance to the toxicants and tissue susceptibility. Because the kidney organ is appears to be the critical organ of heavy metal toxicity for the ingestion of mercury ions. During the heavy metal treatment the accumulation mercury and its toxicity effect is pronounced mainly in the kidney tissues to release more and more amount of LPO content was liberated (Vanithasri and Jagadeesan, 2013). The release of
Lipid peroxidation is mainly initiated by free radicals and is the oxidative deterioration of poly unsaturated fatty acids which is synthesized in intoxicated animals (Jagadeesan and Sankarsami Pillai, 2007; Mahboob et al., 2001; Sakaguchi and Yokota, 1995). The significant increase in lipid peroxidation was found in kidney tissue of rat when treated with mercury ions (vide in chapter 4) can act as potent pro-oxidant in kidney cortical cells, causing depolarization of mitochondrial inner membrane followed by hydrogen peroxide production by the mitochondrial electron transport chain and concomitant increase in iron dependent LPO (Kavitha and Jagadeesan, 2006; Bharathi et al., 2012). Kidney damage induced by HgCl$_2$ generally reflects disturbances and damages occurred in the kidney cells, which leads to not only characteristic changes of antioxidant enzyme activities but also inhibit its synthesis. The increased levels of LPO may be interpreted as a result of the kidney cell destruction or changes in the membrane permeability. An enhanced LPO content are characteristic of kidney damage, therefore their release into the serum confirmed the HgCl$_2$-induced kidney damage (Vanithasri and Jagadeesan, 2013).

During the recovery period, HgCl$_2$ followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract the kidney sections shows a complete regeneration of its histoarchitecture. The newly regenerated renal cells are presented in all regions. The size and shape of the renal cells and glomerulus cells are restored. Kidney tissue of rat fed with Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract alone shows a remarkable increase in the size, and shape of the nephran cells that are regularly arranged respectively. Similar types of results were also observed by Kavitha (2004) in mercury intoxicated mice when
treated with selenium and *Tribulus terrestris* fruit extract respectively. She also suggested that the alteration of cellular arrangement and its size and shape has retained its normal level in the kidney tissue of *Mus musculus*, when treated with selenium and MF of *Tribulus terrestris* fruit extract respectively. Moreover, it protects the animals from HgCl$_2$ induced nephrotoxicity and executes its modulating the histopathological and biochemical changes in during the free radical production. This experimental work suggested that treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract have a preventive and protective effect of the animal and human system from mercuric chloride intoxication rat. Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract can be developed as compound and plant extract against mercuric chloride related disabilities in the future. The histological observations of the present experimental study clearly showed Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract modulated most of the electrophysiological, biochemical and histopathological parameters were maintained to normal status in mercury intoxicated rats, suggesting the beneficial action of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract as a protective agent.