1. GENERAL INTRODUCTION

Now a days all living beings are facing the pollution threat. For environmental biologists pollution is one of the challenging problems as varieties of toxicants have potentially harmful effects on the biological organisms (Kavitha and Jagadeesan, 2003; Sankarsami pillai and Jagadeesan, 2005) including human beings. Hence, human activities play a major role in polluting the environment by introduction of toxic heavy metal compounds (Jagadeesan, 1994; Migliore et al., 1999; Jagadeesan and Sankarsami pillai, 2007). Among the heavy metals, Mercury and its compounds are having high metal toxicity in broad environmental and industrial pollution which produced various diseases or defects both in human beings and animals (Jagadeesan, 2004). Recently, more attention has been given to the toxic effects of mercury and its compounds on the cardiovascular system and the association with hypertension, carotid atherosclerosis, myocardial infarction and coronary heart disease (Virtanen et al., 2005). Chronic mercury exposure reduces the development of myocardial force and inhibition of myosin ATPase activity in the cardiovascular system (Moreira et al., 2003; Wiggers et al., 2008). Moreover, chronic mercury exposure increased vascular resistance and induced hypertension (Houston, 2007). Mercury and its compounds toxic effects on the cardiovascular system is not fully elucidated, but this mechanism is believed to involve an increase in oxidative stress.

Cardiovascular diseases

Today cardiovascular diseases (CVD) are leading cause of death more than of 80% in the world. Cardiovascular disease is the most common cause of death in India including coronary heart disease and myocardial infarction (MI).
The statistical data suggests that by the year 2020, India will have the largest CVD burden in the world. It is predicted that CVD will be the most important cause of mortality in India by 2020 (Rajdurai and Prince, 2007).

Cardiovascular diseases are also known as heart and circulatory diseases because it mainly affects the heart and blood circulatory system. It is also related to renal disease and hepatic disease directly or indirectly affected to oxidative damage in a common mechanism of molecular and cells damaged to the organs (Deavall et al., 2012). CVD is caused by a granular build up of more fatty acid on the walls of coronary arteries. This causes the artery to narrow down and marks it harder for the artery to supply heart muscle with blood and oxygen (Marshall and Davies, 1999). The high levels of blood circulation cholesterol level accumulation in cardiac tissue are well associated with cardiac tissue damage. Moreover, the lipoprotein levels are also altered in intoxicated animals (Raj et al., 2010). Thus, the lipids and lipoproteins play an important role in the pathogenesis of myocardial infarction (Sharmila and Rajdurai, 2012). It occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue (Upaganlawar et al., 2012). Hence, myocardial infarction is an acute condition of necrosis of myocardium that occurs as a result of imbalance between coronary blood supplies to any part of heart, resulting in death of cardiac tissue (Radhiga et al., 2012). Further, the myocardial infarction in lipid peroxidation content and total cholesterol level increased cardiac tissue damage. Heavy metal exposure has been linked to increased incidence of cardiovascular diseases (Vijayakumar et al, 2014). Therefore, Heavy metal toxicity also promotes heart disease in animals and it is a leading cause of death. The heavy metals induced by cardiotoxicity studies have shown a number of risk factors for cardiotoxicity including oxidative stress.
Mortality of cardiovascular disease in India

According to the Gupta et al. (2008) statement the cardiovascular disease in WHO has predicted that from years 2000 to 2020 disability-adjusted life years lost in India shall double in both men and women from 7.7 and 5.5 million, respectively. Punjab (49%), Goa (42%), Tamil Nadu (36%) and Andhra Pradesh (31%) have the highest myocardial infarction related mortality estimates (Gupta et al., 2006). State-wise differences are correlated with prevalence of specific dietary risk factors in the states (Rastogi et al., 2004).

Cardiovascular disease affected more people and causes more death each and every year. The following types of cardiovascular problems lead to heart failure in most of the cases. Types of cardiovascular problems include hypertension, coronary heart disease and myocardial infarction, etc.

**Figure 1:** Heart damaged in cardiotoxicity

**Hypertension**

Hypertension is the common cardiac disease followed by ischemic heart disease (Begum and Akhter, 2007). Recently, more attention has been given to the toxic effects of mercury on the cardiovascular system and the association with hypertension, carotid atherosclerosis, myocardial infarction, and coronary heart disease (Virtanen et al., 2005). Moreover, mercury chloride chronic toxic exposure increases vascular resistance and induces hypertension.
Coronary heart disease

In the last 10 years, several reports have focused more attention on the toxic effects of mercury in the cardiovascular system and its association with coronary heart disease (Salonen et al., 2000; Houston, 2007). Previous reports show that the regulatory function of endothelium in coronary vessels is altered by cardiovascular risk factors or disorders such as hypercholesterolaemia, chronic smoking, hypertension and chronic heart failure (Osto et al., 2007). Because mercury exposure is associated with oxidative stress and endothelial dysfunction, more attention has been paid to its toxic effects on the cardiovascular system and its association with myocardial infarction.

Myocardial infarction

Myocardial infarction (MI) is a common cardiovascular disease. MI is one of the leading causes of death in many countries throughout the world. After MI, restoration of cardiac function requires increase of the myofibroblast population, but also a re-vascularization of the injured region (Frangogiannis et al., 2002).

Risk factor of myocardial infarction

Myocardial infarctions induced by different risk factors through environmental pollutions and metal toxicity in particularly by way of mercuric chloride. The death rate of myocardial infarction disease can be explained by the fact there are high levels of standard risk factors and a low level of intervention and metal risk factors. Population based studies have given rise to the concept that specific factors such as genetic, environmental, modifiable and non-modifiable factors are primarily responsible for the increased risk of MI (Oparil and Oberman, 1999). Important
Risk factors include diabetes mellitus, obesity, physical inactivity, oxidative stress, family history and environmental toxicants etc. The most modifiable risk factors are mercuric chloride metal toxicity and environment pollution.

**Hypercholesterolemia**

A high level of cholesterol in the blood is associated with an increased risk of heart attack because cholesterol is the major component of the plaques deposited in arterial walls (Jain *et al*., 2007).

**Triglyceride**

Triglyceride is also a major factor associated risk of myocardial infarction. According to Hopkins *et al.* (2005) increased triglyceride content are independently associated with coronary disease incidence.

**Heavy metal**

Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface water. Anthropogenic activities do, however, cause an increased discharge of these metals into natural aquatic ecosystems (Abdel-Warith *et al*., 2011). Among the heavy metals mercury and its compounds are one of the most dangerous xenobiotic toxicants. It is widely considered one of the most toxic substances on earth (Clarkson, 1997; Sura *et al*., 2011) because elevated concentrations can cause toxicity, bioaccumulative properties and other deleterious effects on biota, including genetic alterations or mutagenesis (Gray and Hines, 2006).
Mercury (Hg)

Mercury is a silver white fluid trace metal found in igneous and sedimentary rocks and in the form of ore cinnabar (mercury sulfide). Mercury (Hg) is a silvery, liquid metal at room temperature. It is harmful to human beings and advanced creatures (Cheng et al., 2006). It is a naturally occurring element found in air, water and soil. It is a persistent, bioaccumulative toxic pollutant and exists in several different forms which can impact individuals through various routes of exposure. Human and industrial activities, including those that use mercury directly or burn mercury bearing fossil fuels like coal, have increased the amount of mercury deposited in the environment. Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders (Risher and Amler, 2005). Nowadays, large populations worldwide are exposed to relatively low levels of Hg, especially via the use of pesticides in agriculture and of fluorescent light bulbs as well (El-Shenawy and Hassan, 2008). In this context, Hg exists in a wide variety of physical and chemical states, each of which has specific characteristics for target organs (Aleo et al., 2002; Ghosh and Sil, 2008).
Sources of mercury and its compounds

Mercurial compounds in nature

Mercury and its compounds are released into the environment through natural geological processes, such as volcanic eruptions, dissolution and volatilization from rocks, soils and sediments (Goering et al., 2002). Natural activities in general constitute the weathering of rocks, volcanic events and geothermal activity (Nriagu and Becker, 2003).

Mercurial compounds in food

Exposure to mercury may primarily occur by uptake from water and ingestion through the food chain (Agarwal and Behari, 2007). Consumption of fish containing elevated levels of methylmercury (MeHg) is the primary vector of human exposure to this toxin (Choi et al., 2009). Mercury and its compounds have been linked to a variety of negative health impacts in animals (Sankarsami Pillai and Jagadeesan, 2004; 2005; Scheulhammer et al., 2007).

Mercurial compounds in industrial processes

Mercury is released into the environment due to anthropogenic activities which include fossil fuels combustion, waste incineration, metal refining and manufacturing, chloralkali production activities. Such types of activities are responsible for discharge a large amount of mercury into the environment (Jagadeesan, 2004). The main source of mercury pollution is chloride-alkaline industry and mining process (Hassett et al., 2004; Mukherjee et al., 2004; Wu et al., 2006; Jarosinska et al., 2008; Wang et al., 2012). Other industrial facilities, including coal-fired power plants
and facilities for the production of mercury thermometers, fluorescent lamps, batteries and electrical products are also responsible for promoting the mercury toxicity in animals (Yang and Wang, 2008). The recent estimation of global mercury emissions range from 5000 to 8000 metric tons per year (Wang et al., 2012).

Absorption of mercury

Elemental mercury

Elemental mercury (Hg) is found in liquid form, which easily vaporizes at room temperature and is well absorbed (80%) through inhalation (Vijay et al., 2010) and easily passes through pulmonary alveolar membranes and enters the blood (Clarkson, 1997). Mercurous and mercuric salts have also been reported to be absorbed through the skin of animals. Hursh et al. (1989) had estimated that dermal absorption contributes approximately 2.6% of the absorbed mercury following exposure to elemental mercury vapour in the air, the other 97.4% occurs through inhalation.

Inorganic mercury

For inorganic mercuric compounds, absorption via the lungs is low; probably due to deposition of particles in the upper respiratory system and subsequent clearance by the mucociliary escalator (Friberg and Nordberg, 1973). Absorption of inorganic mercurial salt occurs following dissociation of ingested soluble divalent mercuric salts such as mercuric chloride (HgCl₂). Approximately 10% of such compounds are absorbed from the gut (Beate et al., 2010). The absorption of the relatively insoluble mono valent mercurous compounds such as calomel (HgCl₂) is thought to depend on oxidation to the divalent form. Inorganic
mercurials are also absorbed across skin and mucous membranes, as evidenced by urinary excretion of mercury following the dermal application of mercurial ointments and powders (Klaassen, 1990).

**Organic mercury**

Most of an oral dose of methyl mercury (MeHg) is absorbed from the gastrointestinal tract (Beate *et al*., 2010), and about 90% is absorbed from the gut. Dermal and inhalation absorption is known to occur, though precise quantization and exclusion of concomitant absorption by ingestion may be difficult to determine (Elhassani, 1982).

**Toxicity of mercury**

The chemical form of the mercury and its compounds mainly influenced their toxicokinetics and toxicodynamics in animals. While liquid mercury and rarely soluble mercury salts like mercury sulfide (HgS) are less toxic, soluble divalent mercury salts like mercury chloride (HgCl₂) are more toxic. Most toxic are short chain organic mercury compounds and mercury vapour (Beate *et al*., 2010). But according to Pinho *et al*. (2002) mercury in any form is toxic due to its high affinity for lipids. Because, the lipids allow the mercury ion movement across the cell membranes and the toxicity of mercury can interfere with cell metabolism. The major mechanism of mercury toxicity is thought to be the high affinity of Hg²⁺ for sulphhydryl groups, resulting in severe inhibition of critical enzymes essential for different biochemical pathways (Johansson *et al*., 2002), and reactions with the phosphoryl, carboxyl, and amide groups which interrupts cellular metabolism, membrane functions, and protein synthesis.
In the body, metallic mercury is slowly ionized and can, therefore, react with free SH-groups of proteins, or creating protein adducts through modification of side chains leading to changes in protein shape and activity (Agarwal and Behari, 2007).

**Mercury induced organ toxicity**

**Mercury induced Cardiotoxicity**

Chronic exposure of mercuric chloride causes heart failure. Both organic and inorganic mercuric chloride accumulates in the internal organs especially in heart tissue and it has been associated with elevated blood pressure, abnormal heart rhythms and increased heart attack (Furieri et al., 2011). Mercurial compounds are widely used in industries and their hazards to animals have been well documented (Margarat et al., 2001; Kavitha and Jagadeesan, 2003; Jagadeesan, 2004; Sankar Samipillai and Jagadeesan, 2004, 2005). At present, humans are exposed to mercury mostly through consumption of organic mercury-contaminated fishes, the administration of thimerosal in vaccines, and the inhalation of mercury vapor from dental amalgams (Mckelvey et al., 2007). Mercuric chloride (HgCl$_2$) is one of the environmental heavy metals pollution and is create various organ physiological toxicity including cardiac and oxidative stress. Because mercury exposure is associated with oxidative stress and endothelial dysfunction, more attention has been paid to its toxic effects on the cardiovascular system (Houston, 2007; Rizzetti et al., 2013). The harmful effects of mercury during its accumulation in animals are mostly due to the excessive release of reactive oxygen species (ROS), the
increased lipid peroxidation in the cells and reduction of antioxidant defenses, thus inactivating important enzymes that are responsible for body’s defenses, such as glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and in different organs (Rrizzetti et al., 2013; Bharathi et al., 2014; Vijayakumar et al., 2014).

**Mercury induced hepatic-toxicity**

The liver is a major site of metabolism for mercury and it can accumulate in the liver, resulting in severe hepatic damages (Vanithasri and Jagadeesan, 2013; Bharathi et al., 2014). Previous studies have revealed that HgCl₂ caused antioxidant, histopathological and ultrastructural lesions in the liver evidenced by periportal fatty degeneration and cell necrosis (El-Shenawy and Hassan, 2008; Bharathi et al., 2014).

**Mercury induced renal-toxicity**

Exposure to mercury can cause adverse health effects in humans, and the kidney is an important target organ for inorganic mercury. Occupational exposure to elemental mercury has been shown to cause functional changes in the proximal tubular cells, expressed as increased urinary excretion of the low-molecular-weight proteins, enzymes, and even to affect glomerular function (Cardenas et al., 1993; Ellingsen et al., 2000). Some irregularity in renal function in occupational exposure has been observed at lower mercury (Ellingsen et al., 2000; Bharathi et al., 2014).
Role of oxidative stress in myocardial infarction and its nullifying effects

**Figure 3: Effects of oxidative stress and antioxidants**

Even very low levels of chronic mercury exposure promote endothelial dysfunction as a result of increased inflammation, oxidative stress, reduced oxidative defense, reduction in nitric oxide (NO) bioavailability, which increases the risk of cardiovascular disease (Houston, 2011). Mercury chloride has high affinity for sulhydryl (SH) group leading to inactivating numerous enzymatic reactions amino acids and sulphur containing antioxidant (GSH) with subsequent decreased oxidant defence and increased oxidative stress. Mercury induces mitochondrial dysfunction with reduction in antioxidant properties, depletion of glutathione, and increased lipid peroxidation and oxidation stress (Vanithasri and Jagadeesan, 2013).

**Amerlarative agents**

There is no medical treatment to prevent the accumulation of mercury or to eliminate the mercury from the tissues (Jagadeesan, 2004). Chelating therapy is recommended for acute mercury poisoning. The chelator should be administrated immediately within short interval following the mercury intake.
Historically, plants have been used as folk medicine against various types of disease. Remedies from plant sources (Indian system of medicine the ‘Ayurveda’ have proved to be very popular in primary health care in India for a long time. Due to low acceptability and inherent toxicity, chemical agents are avoided against heavy metal toxicity. So, in recent years phyto drugs are used as modulator against heavy metal toxicity (Sharma et al., 2005).

**Ferulic acid**

Ferulic acid is a major constituent of fruits and vegetables such as orange, tomato, carrot, sweet corn, and rice bran (Sribalasubashini et al., 2003). It is one of the phyto drugs which is used for cardiotonic in animals. Ferulic acid is a phenolic compound that exhibits a wide range of therapeutic effects against various diseases, including cardiovascular diseases, cancer, diabetes, hepatic toxicity and neurodegenerative diseases. It possesses three distinctive structural motifs that can possibly contribute to its free radical scavenging capability. The presence of electron donating groups on the benzene ring (3-methoxyl and more importantly 4-hydroxyl) of Ferulic acid gives them additional property of terminating free radical chain reactions. The next functionality-the carboxylic acid group in Ferulic acid with an adjacent unsaturated C–C double bond-can provide additional attack sites for free radicals and thus prevent them from attacking the membrane. In addition, this carboxylic acid group also acts as an anchor of Ferulic acid, by which it binds to the lipid bilayer, providing some protection against lipid peroxidation (Kanaski et al., 2002).
It is believed that the Ferulic acid supplies hydrogen to free radicals with phenolic -OH groups to provide the antioxidation effect. Since Ferulic acid has been demonstrated to exhibit so many pharmacological effects, the understanding of pharmacokinetics of Ferulic acid is useful for designing and dosing regimens in pharmacological studies. Furthermore, the pharmacokinetic profile can contribute to the safety and efficacy of Ferulic acid in clinical applications (Li et al., 2012).

A number of pharmacology studies indicated that Ferulic acid have antioxidant activities.

**Figure 4:** Ferulic acid used in pharmacology

**Terminalia arjuna**

*Terminalia arjuna* family Combretaceae a large tree is found throughout the South Asian region. Phytochemicals are a large group of beneficial chemicals of plant origin (phyto-plant, chemical- compounds) that are found in *Terminalia arjuna* seed extract. The extract of *Terminalia arjuna* seed extract has been pharmacologically used for long year as a potential cardio protective agent (Mythili et al., 2012). *Terminalia arjuna* seed extract has various compounds and which they have wide pharmacological activities (Manna et al., 2008).
The methanolic fraction of *Terminalia arjuna* seed extract contains a very high level of flavonoids. Flavonoids have been detected to exert antioxidant, anti-inflammatory and lipid lowering effect while glycosides are cardiotonic, thus making *Terminalia arjuna* seed extract unique amongst currently used medicinal plants (Doorika and Ananthi, 2012). *Terminalia arjuna* is a famous Indian folk medicinal plant used as cardiotonic in heart failure, ischaemic cardiomyopathy, atherosclerosis and myocardium necrosis (Dinesh et al., 2013). Phytochemical extracts from *Terminalia arjuna* species have been known for their antioxidant and antimicrobial properties. They are used in the management of cardiovascular diseases, myocardial infarction, degenerative neurological diseases, cancer, amyloidosis, acute pancreatitis, arthritis, atherosclerosis, inflammatory bowel disease, diabetes, senile dementia, retinal degeneration and senile cataract particularly in humans owing to their antioxidation potential (Dwivedi and Jauhari, 1997).
Rational for choosing Ferulic acid and methanolic fraction of *Terminalia arjuna* seed extract

Medicinal plants play a key role in human health care from time immemorial. Currently, there is increasing realization that plant products can influence the course of heart disease and its treatment (Dinesh et al., 2013). These plants have been recently considered of interest in myocardial injury because they exhibit a clearance of cardiac toxicity including vasodepressor, cardiotonic, and anti-dys-rhythmic properties. Further, these plant products are investigated for the reasons mentioned below:

- Ferulic acid is ubiquitous triterpenoids in plant kingdom, medicinal herbs, and is integral part of the human diet.
- Ferulic acid is already identified as an active constituent in some cardioprotective plants.
- The methanolic fraction of *Terminalia arjuna* seed extract is also having cardio-protective role
- Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract is relatively non-toxic in nature.
- No systematic experiment has been carried out on the effect of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract by analyzing the whole genome expression on mercury chloride-induced cardiotoxicity.
- Protective effect of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract are cardio- tonic against oxidative stress and cardiovascular disease.
With this point of view, an attempt has been made in the present study to investigate the influence of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract on mercury intoxicated rats, *Rattus norvegicus*. The present programme of experimental work covers the following aspects.

**The objectives of the present studies**

- To observe the histopathological changes in the selected tissues of rats treated with mercuric chloride, followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatments respectively.
- To observe the changes in the level of Lipid peroxidation and antioxidant status of rat treated with mercuric chloride, followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatment respectively.
- To observe the changes in the level of bio-marker enzymes AST, ALT, ALP, CPK and Total cholesterol in the serum of rats treated with mercuric chloride, followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatments respectively.
- To observe the changes in electrocardiogram (ECG) spectrum analysis of rats treated with mercuric chloride, followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatments respectively.
- To observe the immune-histo-chemistry in the expression of cyclooxygenase-2 (COX-2) in the heart tissue of rats treated with mercuric chloride, followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatment respectively.
To observe the changes in the level of inflammatory markers (TNF-α) in the heart tissue of rats treated with mercuric chloride, followed by Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract treatments respectively.

To observe the changes in the level of TGF-β1 in the heart tissue of rats treated with mercuric chloride followed by Ferulic acid and methanolic fractions of *Terminalis arjuna* seed extract treatments respectively.