10. SUMMARY

Now a day 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 including human beings. Hence, Human activities play a major role in polluting the environment by introduction of toxic heavy metal compounds. Among the heavymetals, Mercury and its compounds are having high metal toxicity in board environmental and industrial pollution which produced various diseases or defects both in human beings and animals. 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. Chronic mercury exposure reduces the development of myocardial force and inhibition of myosin ATPase activity in the cardiovascular system. Moreover, chronic mercury exposure increased vascular resistance and induced hypertension. Mercury and its compounds produces toxic effects on the cardiovascular system is not fully elucidated, but this mechanism is believed to involve an increase in oxidative stress.

Historically, plants have been used as folk medicine against various type 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. 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 *Terminalis arjuna* seed extract on mercury intoxicated rats, *Rattus norvegicus*, since little information is available on cardiotoxicity in animals. The present programme of experimental work covers the following aspects.

In the present experimental studies, healthy adult female Wistar rats, *Rattus norvigicus*, were procured, bred and reared in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University.

During the experimental periods animals were kept in the same experimental condition for acclimatisation. At sub-lethal dose metal toxicity tests were conducted to measure the impact of the toxicant on rat upto 45 days. After the scheduled treatments, the animals were sacrificed and the tissues (Heart, liver and kidney) were taken into the biochemical, histopathological and bioenzymological purposes. Wistar albino rats were divided into six groups each consisting of six animals.

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. Histological examination of heart tissue of mercury treated rats showed moderate hypertrophy of cardiomyocytes. 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.
The irregular shape and size of the hepatocytes were noticed in mercury intoxicated liver. 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. 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.

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.

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 from mercuric chloride intoxication. 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.
During the mercury intoxication, the level of LPO content was drastically increased in Heart, Liver and Kidney tissues of rat mainly due to implicate an increased production of free radicals in mercury intoxicated rat. The lipid bilayer of intracellular organelles of the target organs are affinity with free radicals to cause destabilizes especially lysosomal membranes and results in the rupture of lysosomes. The liberation of lysosomic materials can promote the cellular damages in the target organs. It is well known mercury and its compounds cause’s fatty changes in the liver, decrease the activities of anti-peroxidative enzymes (GPx, SOD, CAT etc.,) and increase the content of lipid peroxidation products, which results in organ damages. Inhibition of antioxidant enzymes in mercury intoxicated animal organs are mainly due to the over production of reactive oxygen species.

Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract might prevent the initiation and propagation of lipid peroxidation process by scavenging free radicals through conjugation with glutathione, leading to the control of oxidative damage and cardiac tissue protection.

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 serum.

The damaged organ tissues are possessing high amount of marker enzymes like CK, LDH, ALT, AST and ALP. The level of these marker enzymes served as sensitive index to assess the severity of organ damages particularly heart damages. During the recovery period, administration of Ferulic acid and methanolic fractions
of *Terminalia arjuna* seed extract on mercury intoxicated rats showed 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.

The electrocardiogram (ECG) is a remarkably important tool for the study of cardiac electrophysiology, both in the clinical and in the experimental setting. The present experimental study shows significant alterations of ECG patterns were observed in mercuric chloride intoxicated rats as compared to normal control rats. The characteristic findings were reductions in the QRS complex, R-R intervals, QT interval and prolongation of cardiac cycle. The present study also observed a significant decrease in heart rate. These alterations could be due to the accumulation of cytokines in the cell membrane of injured myocardium. Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract administration on mercury intoxicated rat cardiac ECG spectrum was altered; it indicated that the toxicity effect of HgCl$_2$ was completely withdrawn and promotes the cell membrane to protect the cardiac tissue.

In the present experimental study, the level of TNF- alpha expression was drastically increased in mercury intoxicated heart tissue. This result suggested that an enhanced level of oxidant properties leads to formation of TNF-alpha expression (inflammatory response). During the recovery period, administration of a Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract against cardiac protection in mercury intoxicated heart tissue. The obtained results are
indicated that COX-2 inhibition reduced collagen deposition in the heart tissues after myocardial infarction. This beneficial alteration in the remodeling process probably reduced cardiac tissue stiffness, thus accounting for the improved cardiac function.

Tumour necrosis factor-α (TNF-α) is a proinflammatory cytokine with cardiodepressant properties. Exposure to mercuric chloride resulted in a significant increased in the production of the pro-inflammation cytokine TNF-α which are mediators of the inflammatory response and which are released from different cell types. During the recovery treatment, Ferulic acid and methanolic fractions of *Terminalis arjuna* seed extract on mercury intoxicated rat heart tissue showed decreased in the level of cytokine TNF-α protein expression to near normal level. The result suggested that TNF-α secretion is increased following chronic administration of mercuric chloride could promote the cardiac damages.

During the mercury intoxication, occurrence of inflammation (enhances of TNF alpha) is play an integral role in the pathogenesis of cardiotoxicity events in animals. Initiation and maintenance of systemic and vascular inflammation in intoxicated animals mainly associated with the coronary artery disease which is responsible for enhancement of pro-inflammatory cytokines. During the recovery treatment, Ferulic acid and methanolic fractions of *Terminalis arjuna* seed extract on mercury intoxicated rats showed decreased in the level of mRNA in TGF-β1 gene expression to near normal level. This result suggests that modulation of collagen production and/or degradation by TGF-β1 may contribute to changes in myocardial structure and function.