6. PROTECTIVE EFFECT OF FERULIC ACID AND METHANOLIC FRACTIONS OF *Terminalia arjuna* SEED EXTRACT ON ELECTROCARDIOGRAM SPECTRUM OF MERCURY INTOXICATED RATS

6.1 Introduction

Heart disease is one of the leading causes of animal death (Vijayakumar *et al*., 2014). Most of heavy metal exposures have been linked to increased incidence of cardiovascular diseases. Among the heavy metal, mercury and its compounds are having one of the highly metal toxicity in board environmental and industrial pollution (Jagadeesan, 2004). Consumption of high quantity of mercury-contaminated food (sea food) changes the blood pressure and cardiac autonomic activity (Valera *et al*., 2011). Mercury and its compounds are transition metal and it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides (Flora, *et al*., 2008) in animal when it was exposed. The ROS enhances the subsequent iron of lipid peroxides and the highly reactive hydroxyl radical. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell (Bashandy *et al*., 2011). The formation of (ROS) in cells leads to the formation of radicals in metabolic processes which cause damage to cardiac cells, these harmful effects are controlled by antioxidant defense system in cells which include enzymes such as superoxide dismutase, catalase, glutathione peroxidase (*Erat et al*., 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 (*Wiggers et al*., 2008). With this point of view the present
experimental study has been designed to determine the cardiac productive role of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract against mercury chloride-induced cardio-toxicity in rats. The cardiac damages, electrocardiogram properties and predisposition to heart rhythm disorders can be determined directly from the ECG records.

### 6.2 Observation

#### 6.2.1 The heart rate factions- control animal ECG spectrum

The Heart Rate, R-R Interval (sec), P wave (mV), PR interval (sec), QRS complex (sec) and QT interval (sec) rat, *Rattus norvegicus*, the analysis of electrocardiogram (ECG) spectrum present in the heart Rate factions were 63.16 ± 1.27, 0.010 ± 0.003, 0.010 ± 0.002, 0.72 ± 0.002 and 0.100 ± 0.005 values expressed as n per (second) and (mV). At control treatment for 45 days, the ECG spectrum was drastically normal in heart rate and factions are respectively (Table 9 and Fig. E1)

#### 6.2.2 The heart rate factions-mercury chloride intoxicated rat ECG spectrum

The Heart Rate R-R Interval (sec), P wave (mV), PR interval (sec), QRS complex (sec) and QT interval (sec) rat, *Rattus norvegicus*, the analysis of electrocardiogram (ECG) spectrum present in the heart Rate factions were 57.70 ± 0.86, 0.020 ± 0.007, 0.020 ± 0.007, 0.048 ± 0.007 and 0.156 ± 0.008 values expressed as n per (second) and (mV). The HgCl₂ treatment of at sub-lethal dose treatment for 45 days, the ECG spectrum was drastically increased in heart rate and factions are respectively (Table 9 and Fig. E2).
6.2.3 The heart rate fractions -mercury chloride followed by Ferulic acid treated ECG spectrum

Recovery treatment animal is mercury intoxicated rats. During the treatment of mercury chloride followed by Ferulic acid, The heart Rate R-R Interval (sec), P wave (mV), PR interval (sec), QRS complex (sec) and QT interval (sec) rat, *Rattus norvegicus*, the analysis of electrocardiogram (ECG) spectrum present in the heart Rate factions were 60.00 ± 0.46, 0.016 ± 0.002, 0.020 ± 0.005, 0.052 ± 0.003 and 0.120 ± 0.007 values expressed as n per (second) and (mV). The mercuric chloride followed by Ferulic acid of at sub-lethal dose treatment for 90 days, the ECG spectrum was drastically increased in heart rate and factions are respectively (Table 9 and Fig. E3)

6.2.4 The heart rate factions-mercury chloride followed by methanolic fractions of *Terminalia arjuna* seed extract treated ECG spectrum

Recovery treatment animal is mercury intoxicated rats. During in the treatment of mercury chloride followed by methanolic fractions of *Terminalia arjuna* seed extract, The heart Rate R-R Interval (sec), P wave (mV), PR interval (sec), QRS complex (sec) and QT interval (sec) rat, *Rattus norvegicus*, the analysis of electrocardiogram (ECG) spectrum present in the heart Rate factions were 72.12 ± 0.54, 0.008 ± 0.002, 0.010 ± 0.003, 0.040 ± 0.004 and 0.072 ± 0.007 values expressed as n per (second) and (mV). The mercuric chloride followed by methanolic fractions of *Terminalia arjuna* seed extract of at sub-lethal dose treatment for 90 days, the ECG spectrum was drastically normal in heart rate and factions are respectively (Table 9 and Fig. E4)
6.2.5 The heart rate factions- Ferulic acid alone treated ECG spectrum

Ferulic acid is alone treatment followed animal. The heart Rate R-R Interval (sec), P wave (mV), PR interval (sec), QRS complex (sec) and QT interval (sec) rat, *Rattus norvegicus*, the analysis of electrocardiogram (ECG) spectrum present in the heart Rate factions were 68.18 ± 0.73, 0.012 ± 0.002, 0.012 ± 0.002, 0.048 ± 0.007 and 0.080 ± 0.002 values expressed as n per (second) and (mV). The Ferulic acid alone treatment for 45 days, the ECG spectrum was drastically increased in heart rate and factions are respectively (Table 9 and Fig. E5).

6.2.6 The heart rate factions- methanolic fractions of *Terminalia arjuna* seed extract alone treated ECG spectrum

Methanolic fractions of *Terminalia arjuna* seed extract is alone treatment followed animal. The heart Rate R-R Interval (sec), P wave (mV), PR interval (sec), QRS complex (sec) and QT interval (sec) rat, *Rattus norvegicus*, the analysis of electrocardiogram (ECG) spectrum present in the heart Rate factions were 75.00 ± 0.21, 0.030 ± 0.004, 0.004 ± 0.004, 0.032 ± 0.002 and 0.080 ± 0.005 values expressed as n per (second) and (mV). The methanolic fractions of *Terminalia arjuna* seed extract alone treatment for 45 days, the ECG spectrum was drastically increased in heart rate and factions are respectively (Table 9 and Fig. E6).
6.3 Discussion

The electrocardiogram (ECG) is a remarkably important tool for the study of cardiac electrophysiology, both in the clinical and in the experimental setting. It is known that the rat constitutes an important model for cardiovascular physiology research and, for long, ECG-based studies have been conducted in this animal model. Electrocardiograph is generally used for the definite diagnosis of myocardial infarction through their abnormalities of heart function (Peacock et al., 2007). The present experimental study shows significant alterations of ECG patterns 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 (Vide in chapter 7 & 8). Mercury and its compounds may promote atherosclerosis and hence increase the risk of myocardial infarction in several ways including the ability of heart muscle to contract and its electrical conductivity and regulations of cardiac activity (Salonen et al., 1995). Some researchers also found that mercury and its compounds also promote the MI was evident from ECG changes and marker enzymes alterations when it accumulates its toxicity in cardiac tissues (Vassallo et al., 2011).

Spectral analyses of ECG are very useful tool for identifying the myocardial infarction. During the mercury treatment, the raise of ST segment was noticed in the ECG spectrum of mercury intoxicated cardiac tissue when compared to normal untreated rats. Further, the spectrum of ECG shows lesser intensity of P
wavelength, QRS complex were smaller than the normal rat ECG spectrum, and reducing the R-R and QT intervals were also noticed in mercury intoxicated cardiac tissue in continue for long time. Increasing the heart rate and alteration of ECG spectrum are mainly due to the damage of myocardial cell membrane (O’Neil et al., 1960). An enhanced level of oxidant properties in cardiac tissue (vide in chapter 4) leads the cell membrane damage by the way of altering the ECG spectrum (Vijayakumar et al., 2014). Similar types of results were also observed by Ahmed et al. (2013) in lead induced cardiotoxicity in rats. They are suggested that the accumulation of lead toxicity promote the cardiotoxicity through the enhanced level of oxidant properties and simultaneously decreased level of antioxidant properties in rats. The present experimental study also confirmed the results.

Heart rate (R-R intervals) is the speed of the heartbeat measured by the number of heartbeats per unit of time typically beats per minute (bpm). The heart rate can vary according to the body's physical needs, including the need to absorb oxygen and excrete carbon dioxide. Activities that can provoke change include physical exercise, sleep, anxiety, stress, illness, ingesting, and drugs. Due to the toxicity effect the heart rate will be changed. Present experimental study also showed significant alteration in ECG patterns of mercuric chloride in intoxicated rat. These alterations could be due to the consecutive loss of cell membrane potential in injured in cardiac tissue or myocardium. Similar types of results were also observed by Gandhi et al. (2013) in doxorubicin induced cardiotoxicity in rats. They are explained the ECG examination to verify the marred cardiac contractility and conduction after doxorubicin intoxication in rats.
During recovery treatment, the raise of ST segment was noticed in the ECG spectrum of mercury intoxicated cardiac tissue when compared to normal untreated rats. During the recovery period, elevated levels of ST segments were drastically reduced to the normal level. The result suggested that the administration of Ferulic acid on mercuric chloride intoxicated rat can promote the protective effect on myocardial infarction. 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 study, the figure2 clearly showed an elevation of QT intervals and P wave length in mercury intoxicated rat and post treatment with Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract markedly inhibited mercury induced QT intervals and P wave length elevation suggestive of its cell membrane protecting effects (fig E3 & E4). The appearances of QT segment elevation are some of the indicative signs of ischemia. The prominent QT and RR interval were seen only on severe ischemia, infarction and in animals with severe heart diseases. The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by reduced levels of RR intervals in mercury intoxicated animals. Post treatment of Ferulic acid and methanolic fractions of *Terminalia arjuna* seed extract showed a protective effect against mercury induced altered ECG pattern and eliminated the acute fatal complications by protecting the cell membrane damage. Similar types of results were also observed by Gandhi *et al.* (2013) in doxorubicin induced cardiotoxicity in rats when treated with Felodipine pretreatment. Further, they are suggested that abnormalities seen in the ECG after doxorubicin treatment were normalized to a significant extent (ST interval normalization was significant at $P < 0.01$)
in felodipine treated rats. Khatib et al. (2011) have also been observed the similar
type of results in ISO treated rats when administration with aqueous extract of
Punica granatum flowers. They are suggested that pre treatment with aqueous
extract of Punica granatum flowers decreased the markers enzymes and
normalized the ECG pattern in ISO induced myocardial infraction rats.

Findings of the present study suggest that the administration of Ferulic acid
and methanolic fractions of Terminalia arjuna seed extract is protected the
cardiovascular disease from HgCl₂ intoxicated myocardial infarction. The data can
clearly demonstrate that the normal and experimental groups also modulated the
ECG spectrum analysis in cardiotoxicity. The present experimental results strongly
suggest that the administration of Ferulic acid and methanolic fractions of
Terminalia arjuna seed extract has a beneficial effect and protects the structural
and functional alterations made in myocardial infraction.