cardiac injury & ischemic markers
Chapter 3

Cardiac injury and Ischemic markers

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Results

Plasma levels of lactate dehydrogenase (LDH) and creatine kinase (CK-MB) were determined by the method outlined in the commercial assay kits (DIALAB Produktion und Vertrieb von chemisch-technischen, Austria). We observed significantly higher plasma LDH level in the ISO group compared with the control. There was a significant lowering of the plasma LDH level in flavonoid rich AIE (53.09%) and amlodipine (50.53%) treated groups compared with the ISO. Flavonoid rich AIE alone treated rats did not show any significant alterations in the plasma LDH level compared to control rats.

Rats treated with AIE (86.0 ± 6.95) did not show any significant (P<0.05) effect in plasma CK-MB. Rats induced with ISO showed significant (P<0.05) increased activity of serum CK-MB (223.66 ± 5.79) compared to normal control rats (82.21 ± 4.37). Pretreatment with flavonoids rich AIE (200 mg/kg) and amlodipine (9 mg/kg) significantly decreased (53.09% and 42.09% respectively) the activity of this enzyme in the plasma of ISO induced rats compared to ISO alone induced rats. The values are expressed in IU/L.

The levels of ischemia modified albumin was determined by the method of Arzu et al. (2008). ISO treated rats showed significantly increased IMA compared to control group. Therapeutic and prophylactic treatment with both flavonoid rich AIE (19.02%) and amlodipine (13.88%) showed significant reduction in IMA levels compared with the ISO group. Administration of AIE alone did not produce any significant change in IMA measurement compared to control group. Atherogenic index of plasma (AIP) was calculated as log (TG/HDL-C). Flavonoid rich AIE pretreatment
significantly decreased AIP when compared to the ISO group which exhibited significant hike in the AIP by 105.12% compared to the control group. The effect of AIE reduction of hike in AIP was more prominent than that of amlodipine.

Non-HDL-C is calculated as total cholesterol minus HDL-C. ISO treated rats registered a significant increment in non-HDL-C levels of plasma (146.07%) compared to control rats. However, AIE and amlodipine pretreated rats showed a lesser increment of 44.98% & 38.21% for non-HDL-C. Control rats treated with AIE alone did not register any alterations in the plasma levels of non-HDL-C compared to control rats.

There was a significant increase in LDL/HDL ratio in the ISO treated group compared with the control. The LDL/HDL ratio was significantly reduced in AIE 200 mg/kg (63.92%) and amlodipine 9.0 mg/kg (61.05%) pretreated groups compared with the ISO group. There was no significant alteration in AIE alone treated groups compared with the control.

Pathological Q-waves along with ST-segment elevation was recorded and used as the index of ischemic severity. In anesthetized rats, ISO induced ST-elevation, indicating a markedly ischemia as compared with normal rats (control group). The ISO induced ischemia was inhibited by flavonoid rich AIE. On the basis of ST segment elevation presented in Figure 3.7, AIE reduced ISO induced ST-elevation with marked alteration as compared to amlodipine treated rats.

Discussion

Cytosolic enzyme LDH serves as a marker of myocardial injury that leaks out from the damaged tissues to the blood stream when there is rupture of cell membrane which in turn leads to increase in the permeability of cell membrane (Wang et al., 2009). Hence, the amount of this cellular marker reflects membrane integrity and/or permeability. LDH has been used traditionally as a nonspecific diagnostic tool for myocardial ischemia.

A rise in the proportion of LDH in the plasma can be diagnostic of myocardial ischemia (Nigam, 2007). LDH begins to rise in 12–24 h following MI and peaks in 2–3 days gradually dissipating in 5–14 days. The increased levels of plasma LDH in ISO-induced rats could be due to ISO-induced cardiac necrosis (Yogeeta et al., 2006). Tissue hypoxia caused by ISO has been reported to result in
increased cell permeability and damage leading to leakage of myocardial LDH into plasma. This leakage is due to non-specific alteration in the integrity plasma membrane and/or permeability of myocytes (Padmanabhan and Mainzen, 2006). Significantly elevated activity levels of plasma LDH in our ISO treated rats are in accordance with previous reports (Thounaojam et al., 2011; Wang et al., 2009), which indicates lesion of the myocardial membrane.

The concurrent treatment with flavonoid rich AIE significantly lowered the ISO induced elevation of serum LDH level. It demonstrates that AIE could maintain membrane integrity, thereby restricting the leakage of this enzyme. This could be due to the free radical scavenging property of the extract in the presence of antioxidative phytochemicals such as flavonoids and flavonoid glycosides (Rastogi and Mehrotra, 1991). Numerous experimental and clinical studies have reported that amlodipine ameliorates myocardial injury and SOX. Hence, we used this drug as a standard (Umemoto et al., 2004; Kang et al., 2009). The results of our study show that amlodipine also alters LDH levels. AIE may be correlated with amlodipine, a Ca\(^{2+}\) channel blockers having similar pharmacological benefits.

Creatine kinase (CK-MB) is an enzyme capable of reversibly transferring a phosphate group from the energy storage form of creatine phosphate to a molecule of ADP, producing ATP (Rosalki et al., 2004). CK-MB is localized predominantly in the heart and this makes it a valuable diagnostic tool for MI since damage specific to the myocardium would result in elevation of CK-MB levels (Loh et al., 2007; Rosalki et al., 2004; Libby, 2001). CK-MB estimation is considered as the standard to which all cardiac biomarkers will be compared (Christensen and Azzazy, 1998). When myocardial cells containing creatine kinase (a myocardial tissue injury marker) are damaged, the cell membrane becomes permeable which results in the leakage of enzyme into the blood stream.

The amount of these cellular enzymes obtained in plasma reflects the changes in plasma membrane integrity and/or permeability. Our results showed significant elevation in the levels of CK-MB in serum of ISO-injected rats, which were in line with the previous reports (Zhou et al., 2008; Bertinchant et al., 2000) and were an indication of ISO-induced necrotic damage of the myocardial membrane. CK-MB assays, the cardiac damage markers of MI, also have high sensitivity and specificity. The increased
activity of plasma CK-MB observed in ISO induced rats might be due to cardiac damage induced by ISO (Ahmed et al., 2004). Pretreatment with flavonoid rich AIE seems to preserve structural and functional integrity of the myocardial membrane, which lowered the activity of CK-MB and level in the hearts of ISO-induced rats to near normal. This might be due to the protective effect of flavonoid rich AIE on the heart, which reduced the extent of cardiac damage induced by ISO and thereby restricted the leakage of these products from the myocardium tissue when compared with the ISO group. Amlodipine treatment also showed a similar effect in line with earlier reports (Zhou et al., 2008).

MI is the functional definition of a clinical syndrome with a heterogeneous underlying etiology, but has a common manifestation characterized by the exhaustion of the reserve force of the heart (Micheletti et al., 1995). In the last decade, several evidences suggested that SOX and inflammation might be involved in the pathogenic processes in HF (Mallat et al., 1998; Belch et al., 1999; Ide et al., 2000). ISO in large doses produce myocardial necrosis. Since ISO readily undergoes oxidation and the oxidation products of catecholamine are responsible for myocardial damage. ISO have been shown to enhance myocardial oxygen consumption and enhance the extents of myocardial damage during evolving MI. Higher ISO levels within first few hours of MI subsequently appeared to have greater myocardial damage and higher mortality rates support the concept that ISO may in themselves exert a deleterious effect.

The heart is more dependent on a continuous supply of oxygen than are most other tissues of the body since short periods of anoxia during MI result in loss of contractility and irreversible damage to myocardial tissue (Sudhira and Nargis, 2007). Free radicals are the key mediators associated with MI (Wattanapitayakul and Bauer, 2001). They are formed by infiltration of white cells into ischemic myocardium. ISO, upon oxidation increases lipid peroxidation through enhanced free radical formation and causes oxidative severe stress in the myocardium resulting in infarct like necrosis of the heart muscles (Chattopadhyay et al., 2003; Rajadurai and Prince, 2007). In addition, bad eating habits, sedentary lifestyle, and stressful life, the risk factors for IHDs exacerbate the state of imbalance between the free radicals and the antioxidant defenses contributing to the structural and functional changes of some proteins, such as the human serum albumin (HSA), which plays a vital role in the efficient antioxidant
defense of the organism (Bourdon et al., 1999). Overproduction of free radicals may modify the N-terminal region of HSA generating ischemia-modified albumin (IMA) (Duarte et al., 2009; Kaefer et al., 2010; Gottlieb et al., 2010; Bhagavan et al., 2009).

Enormous amount of reactive oxygen species (ROS) like superoxide, hydrogen peroxide and hydroxyl radical are produced during MI (Rajadurai and Stanley, 2006). These free radicals affect the albumin ability to combine with metals such as cobalt, and may promote an increase of IMA levels (Kaefer et al., 2010). Lipid peroxidation often occurs in response to enhanced SOX, and MDA is one of the low-molecular weight end products formed via decomposition of certain primary and secondary products of cell membrane injury due to lipid peroxidation. It is known that HSA is the primary binder of fatty acids, commonly known as FFA, and that plasma concentrations of such acids are increased in ischemia. Bhagavan et al., (2009) described that changes in IMA values during acute muscular heart tissue necrosis are likely to be caused by reversible conformational changes in HSA associated with FFA fluxes.

Ideally, it is essential to identify myocardial ischemia before the onset of irreparable myocardial cell damage. Thus, identification of a biochemical marker that is sensitive and specific for myocardial ischemia and can be rapidly measured in serum would be clinically valuable. Serum based biochemical test has been found to be useful in the diagnosis of acute myocardial ischemia (Bar-Or et al., 2000). The basic principle of this test involves the N-terminal region of human serum albumin (HSA) and its inherent affinity for the metal ion, Co (II). Serum albumin of individuals with myocardial ischemia exhibits reduced binding to Co (II) compared with serum albumin of nonischemic individuals. This reduced binding of Co (II) to serum albumin has also been observed in individuals with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty (Bar-Or et al., 2001).

In the present study, ISO induced myocardial ischemia was associated with a significant increase in IMA levels in serum, which were in agreement with previous study (Mecit et al., 2011). The present study determined the significance of ischemia-modified plasma albumin in the early diagnosis of myocardial ischemia in an experimental model. Flavonoid rich AIE pretreatment showed a significant reduction in IMA level, suggesting that AIE reduces oxidative stress thereby preventing the generation of free radicals in accordance with earlier studies (Rebai et al., 2009; Bors et
The most important natural phenolics are flavonoids because of their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties (Kahkonen et al., 1999). In fact, flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation (Williams et al., 2004). These compounds, which are widely distributed across the plant kingdom, represent the most abundant antioxidants in the diet and they have gained tremendous interest as potential therapeutic agents against a wide variety of diseases, most of which involve oxidant damage (Ross and Kasum, 2002). These findings indicated that the anti-ischemic potential of AIE can be attributed to its antioxidant activity, at least in part.

Atherogenic lipoprotein profile is also an important risk factor for ischemia, the higher the value, the higher the risk of developing IHD and vice versa (Martirosyan et al, 2007; Brehm et al., 2004; Frohlich and Dobiasov, 2003; Dobiasov, 2004). It is characterized by high ratio of LDL-C to HDL-C and increased level of TGs. Predominance in plasma of small dense LDL-C and small HDL-C particles is associated with an increased risk of ischemia while large HDL-C particles are associated with decreased risk. TGs were of prognostic significance and are known to contribute to ischemic risk with high LDL-C/HDL-C ratio, when used in a ratio with HDL-C become an excellent predictor of likelihood of future myocardial ischemia. AIP has a place in the assessment of lipid-related risk for ischemia as it is derived from more precise measurements of atherogenic lipoprotein profile (Dobiasova and Frohlich, 2001). On the basis that people with high AIP have a higher risk IHD than those with low AIP.

Plasma atherogenicity index (AIP) is positively correlated with the fractional esterification rate of HDL (FER\text{HDL}), and that AIP is inversely correlated with LDL particle size. Because FER\text{HDL} predicts particle size in HDL and LDL, which in turn predicts ischemic risk, the simultaneous use of TGs and HDL-C (both readily available in a plasma lipoprotein profile) as AIP may be useful in predicting plasma atherogenicity (Meng et al., 2004). Earlier studies reported that consumption of flavonoids is associated with protection against atherosclerosis in ischemia. This observation is supported by experimental studies in diverse animal models of atherosclerosis showing that flavonoids can inhibit the ischemic disease (Graf et al.,
In this study, we observed that the flavonoid rich AIE, significantly reduced atherogenic index of plasma (AIP) of the ISO treated animals than those of the amlodipine treated and control rats has likely been due to antioxidative and anti-inflammatory properties other than the effect of the extract on plasma lipoproteins. Low atherogenic indices are protective against ischemia (Usoro et al., 2006).

Ischemia and related conditions (metabolic syndrome, obesity, and diabetes) often have elevated triglycerides, low HDL-C, and relatively normal calculated LDL-C values. Relying on LDL-C targets alone can be misleading in such cases, since they produce highly atherogenic VLDL-C and intermediate density lipoproteins (IDLs) as well as small dense atherogenic LDL-C particles, in spite of the normal LDL values. LDL measurement can be very misleading when the TGs are in the (200-400 mg/dl) range. Sustained hyperglyceridemia eventuates in elevated levels of VLDL-C, IDL, and abnormal highly atherogenic LDL-C particles. Stated otherwise, non-HDL cholesterol may be a stronger predictor of coronary risk than LDL-C or TGs in certain populations, since it reflects the sum of serum cholesterol carried by all of the potentially atherogenic lipoproteins LDL-C, VLDLC, IDL and other remnant lipoproteins. Moreover, since it is calculated from total cholesterol and HDL cholesterol, both of which are measured directly, it is not affected by the TG level and does not require a fasting specimen.

Non-HDL cholesterol provides a single index of all these apolipoprotein B-containing lipoproteins, essentially acting as a surrogate for direct apo B determinations. The bottom line is that the measurement of LDL-C alone is not an adequate measure of atherogenic risk in ischemia (Ervin et al., 2009). The usefulness of non-HDL-C in the prevention of CVD was confirmed in numerous clinical trials (Liu et al., 2005). Ruminska et al., (2010) evaluated the usefulness of non-HDL-C in the lipid disorders among myocardial infracted obese individuals. It is a cost effective screening test that can be included in ischemic risk profile. Tauheed et al., (2003) suggested its possible involvement in the disease and determined levels of non-HDL-C in patients with IHD. AIE significantly decreased the non-HDL-C in plasma of ISO induced experimental myocardial ischemic rats when compared to the ISO+AML treated rats. The hypolipidemic activity of the flavonoids may be due to a lower rate of lipogenesis and higher rate of degradation (Andrikopoulos et al., 2002; Nichols et al., 2011). High
levels of non-LDL-C show a positive correlation with myocardial ischemia (Grundy, 2002). Therefore, the reduction in the non-HDL-C, the secondary lipid lowering targets further corroborated the school of thought that the extract has antiischemic effect.

The importance of both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels for identification of individuals at increased risk of coronary heart disease (CHD) is now well established. Lipid parameters are important independent risk factors for future ischemic events (Jacobs et al., 1990; Freedman et al., 1994). Regarding the traditional fasting plasma lipid profile-triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, there is no universal acceptance of how this information should be used and interpreted, although several consensus documents have been produced (Fodor et al., 2000; Assmann et al., 1999). There is overwhelming evidence, that an elevated LDL-C concentration in plasma in ischemic conditions, whereas a high HDL-C level is cardioprotective (Isabelle et al., 2001).

Moreover, traditional cholesterol measurements tend to be most accurate at predicting risk for those at the lower and higher ends of the risk spectrum. These measurements are less helpful for the majority of people whose risk falls somewhere in between (Gotto et al., 2000). The measurement and interpretation of LDL-C and HDL-C levels can be emphasized for a better risk predictor. In addition, the number of atherogenic versus non-atherogenic lipoproteins transported in blood provides a more comprehensive evaluation of myocardial risk. Recently, LDL-C/HDL-C ratio has been proven to be an accurate predictor of ischemic risk, which can be obtained from a standard lipid profile and is more accurate than LDL-C or HDL-C alone (Manninen et al., 1992). Changes in ratios have been shown to be better indicators of successful IHD risk reduction than changes in absolute levels of lipids or lipoproteins (Natarajan et al., 2003; Kannel, 2005).

Moreover, while not perfect, measurements of apo B and apo A-1 also tend to reflect the levels of LDL-C and HDL-C (Waldius and Jungner, 2005). An LDL-C/HDL-C ratio point for initiating lipid lowering therapy was determined in our study. Hyperlipidaemia is considered as a major risk factor in the progression of coronary atherosclerosis and is associated with an increase in the incidence of MI (Smith et al., 1992). Furthermore, it was seen in ISO treated rats that might be due to
increased mobilization of lipids from adipose tissue (Palanisamy and Kodukkur, 2010). Results showcased in our study, indicated that flavonoid rich AIE pretreatment improved LDL/HDL ratio in ISO induced MI by effective lipid lowering potential than amlodipine.

Electrocardiographic abnormalities are the main criteria used for the definite diagnosis of MI. ST segment elevation was observed in patients with acute MI and in ISO induced MI in rat (Peacock et al., 2007; Rajadurai and Prince, 2007). Present study showed significant alterations in ST segment ECG patterns in ISO injected rats. These alterations could be due to the consecutive loss of cell membrane potential in injured myocardium. Increased ST segment reflects a potential difference in boundary between ischemic and non ischemic zones and consecutive loss of cell membrane functions in the regional ischemic myocardium (Andre, 2000).

Pretreatment with flavonoid rich fraction of AIE in ISO injected rats showed a significant decrease in ST elevation. In the same context Fard et al., (2008); Upaganlawar and Balaraman (2010) reported that Lagenaria siceraria and prevented ECG ST-segment elevation in myocardial ischemic rats. Flavonoids of Acalypha indica prevented ECG abnormalities and it might be due to the protective effect on heart that reduced the extent of cardiac membrane damage and thus it is evident from the above results that flavonoids rich AIE collectively contributing to its overall antiischemic activity.
Table 3.1: Effect of flavonoid rich AIE on cardiac injury and ischemic markers in the control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I CON</th>
<th>Group II CON+AIE</th>
<th>Group III ISO</th>
<th>Group IV ISO+AIE</th>
<th>Group V ISO+AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH %</td>
<td>76.84±3.39</td>
<td>80.21±3.32</td>
<td>170.74±4.97</td>
<td>82.04±3.77</td>
<td>84.46±4.76</td>
</tr>
<tr>
<td>CK-MB %</td>
<td>82.21±4.37</td>
<td>86.00±6.95</td>
<td>223.66±5.79</td>
<td>104.90±7.14</td>
<td>129.61±8.14</td>
</tr>
<tr>
<td>IMA %</td>
<td>0.520±0.04</td>
<td>0.553±0.05</td>
<td>0.720±0.08</td>
<td>0.583±0.03</td>
<td>0.620±0.06</td>
</tr>
<tr>
<td>AIP %</td>
<td>-0.268±0.05</td>
<td>-0.273±0.02</td>
<td>0.014±0.04</td>
<td>-0.218±0.07</td>
<td>-0.239±0.02</td>
</tr>
<tr>
<td>non-HDL-C %</td>
<td>31.96±8.54</td>
<td>31.21±5.86</td>
<td>76.80±7.68</td>
<td>42.25±6.26</td>
<td>47.40±5.07</td>
</tr>
<tr>
<td>LDL/HDL %</td>
<td>0.512±0.22</td>
<td>0.502±0.15</td>
<td>2.02z±0.18</td>
<td>0.729±0.17</td>
<td>0.787±0.11</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. of 6 individual rats.
Values in the parenthesis are % change (" compared to Group-I, * compared to Group-III)
Values not sharing a common super script differ significantly at *P < 0.05, †P < 0.05, ‡P < 0.05, §P < 0.05 vs control (DMRT).
Fig 3.1: Effect of flavonoid rich AIE on LDH levels in control and experimental animals

Values are mean ± S.D. of 6 individual rats
Values not sharing a common super script differ significantly at \(^aP < 0.05\), \(^bP < 0.05\), \(^cP < 0.05\) vs control (DMRT).

Fig 3.1.1: % change of LDH levels in control and experimental animals

Values are % change ('compared to 'CON', * compared to 'ISO')
Fig 3.2: Effect of flavonoid rich AIE on CK-MB levels in control and experimental animals

Values are mean ± S.D. of 6 individual rats
Values not sharing a common super script differ significantly at aP < 0.05, bP < 0.05, cP < 0.05, dP < 0.05 vs control (DMRT).

Fig 3.2.1: % change of CK-MB levels in control and experimental animals

Values are % change (a compared to 'CON', b compared to 'ISO')
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Fig 3.3: Effect of flavonoid rich AIE on IMA levels in control and experimental animals

Values are mean ± S.D. of 6 individual rats. Values not sharing a common super script differ significantly at \(^aP < 0.05, ^bP < 0.05, ^cP < 0.05\) vs control (DMRT).

Fig 3.3.1: % change of IMA levels in control and experimental animals

Values are % change ("compared to ‘CON’, *compared to ‘ISO’")
Fig 3.4: Effect of flavonoid rich AIP values in control and experimental animals

Values are mean ± S.D. of 6 individual rats
Values not sharing a common super script differ significantly at *P < 0.05, ^P < 0.05 vs control (DMRT).

Fig 3.4.1: % change of AIP values in control and experimental animals

Values are % change (*compared to ‘CON’, ^compared to ‘ISO’)
Fig 3.5: Effect of flavonoid rich AIE on non-HDL-C in control and experimental animals

![Bar chart showing non-HDL-C levels in control and experimental animals.]

Values are mean ± S.D. of 6 individual rats. Values not sharing a common superscript differ significantly at \(^{a}P < 0.05\), \(^{b}P < 0.05\), \(^{c}P < 0.05\) vs control (DMRT).

Fig 3.5.1: % change of non-HDL-C levels in control and experimental animals

![Bar chart showing % change of non-HDL-C levels.]

Values are % change (\(^{*}\)compared to ‘CON’, \(^{#}\)compared to ‘ISO’).
Fig 3.6: Effect of flavonoid rich AIE on LDL/HDL ratio in control and experimental animals

Values are mean ± S.D. of 6 individual rats
Values not sharing a common superscript differ significantly at *P < 0.05, *P < 0.05, ³P < 0.05 vs control (DMRT).

Fig 3.6.1: % change of LDL/HDL ratio in control and experimental animals

Values are % change (*compared to 'CON', * compared to 'ISO')
Fig 3.7: Effect of flavonoid rich AIE on ST-elevation pattern on ECG in control and experimental animals.