plasma & myocardial lipids
Results

Plasma lipids

Table 2.1 represents the plasma lipid profile of control and experimental rats. ISO administered rats showed significant (P<0.05) increase in the levels of TC, LDL-C, VLDL-C, TG, FFA and PL with a significant (P<0.05) decrease in HDL-C. On supplementation with flavonoid rich AIE, the levels of these parameters brought back to normal level compared to ISO alone induced and amlodipine treated rats.

Myocardial lipids

ISO group recorded significant increment (P<0.05) in myocardial TC (38.76%), TG (46.79%), FFA (27.08%) and decrement in PL (-28.60%) compared to CON group. ISO+AIE group recorded significant decrement (P<0.05) in the myocardial tissue TC (-22.67%), TG (-20.58%), FFA (-18.12%) and significant increment in PL (31.68%) compared to ISO group (Table 2.2). Administration of flavonoid rich AIE to normal rats did not have any significant effect on myocardial lipid profile.

Discussion

Plasma lipids

Lipids play an important role in IHDs. Hyperlipidaemia and hypercholesterolaemia are risk factors for development of myocardial ischemia MI. An altered lipid metabolism alters the cardiac function by changing the composition, structure and stability of cellular membranes, which contribute to the cell death that
follows ischemia (Giardano, 2005). ISO treated rats showed increased levels of plasma total cholesterol, TG, LDL-C, VLDL-C, FFA, PL, and decreased level of HDL-C. Our results are in agreement with previous report (Manjula et al., 1992; Prince et al., 2008). Also higher level of LDL has a positive correlation where as high level of HDL has a negative correlation with MI (Buring et al., 1992). Hypercholesterolemia and hypertriglyceridemia were seen in ISO-treated rats which might be due to increased mobilization of lipids from adipose tissue (Palanisamy and Kodukkur, 2010). High level of circulating cholesterol and its accumulation is usually accompanied by ischemia (Mediene-Benchekor et al., 2001).

ISO induces free radical formation which may cause cellular cholesterol accumulation by increasing cholesterol biosynthesis, by decreasing cholesteryl ester hydrolysis and by reducing cholesterol efflux (Deepa and Varalakshmi, 2005). In the present study, flavonoid rich AIE restored total cholesterol level to near normal levels, thereby reducing the risk of MI. This observation is in agreement with the findings of Raju et al., (2008) who reported a positive correlation between *Momordica cymbalaria* extract and cholesterol level. Hypertriglyceridaemia is due to a decrease in the activity of lipoprotein lipase in the myocardium resulting in decreased uptake of triglycerides from circulation. Increased level of TGs is associated with cardiovascular disturbances, and ISO promotes lipolysis in the myocardium.

Enhancement in lipolysis may lead to increased synthesis and secretion of elevated plasma TGs concentration (Sushama et al., 1990). Thus cholesterol and TG lowering properties of AIE could be attributed to hypocholesteromic compounds that may act as inhibitors or activators for some enzymes which participate in cholesterol metabolism or due its ability to suppress lipid peroxidation and may be partially attributed the antiradical activities of the flavonoid components known to act by free radical scavenging or chain breaking mechanism (Zou et al., 2005; Fan et al., 2006). Hypertriglyceridemic patients at a risk for ischemic heart disease often develop a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol, which causes myocardial membrane damage (Paul et al., 2010). Lipoproteins are closely associated with MI. Increased levels of plasma LDL and VLDL-cholesterol fractions along with decreased levels of HDL-cholesterol were observed in ISO-induced rats.
Increased levels of LDL-cholesterol show a positive correlation with MI, whereas HDL-cholesterol levels show a negative correlation. Pretreatment with flavonoid rich AIE to ISO-induced rats minimized the alterations in plasma lipoprotein levels by increasing HDL-cholesterol and decreasing LDL and VLDL-cholesterol levels, which are in agreement with earlier studies (Buring et al., 1992; Rajadurai and Prince, 2006; Monforte et al., 1995; Palanisamy and Kodukkur, 2010). Increased plasma concentration of LDL-C in myocardial ischemic rats may lead to increased rate of entry of LDL particles into the artery wall. While LDL particles are protected from oxidation in plasma by antioxidant compounds, LDL particles trapped within the artery wall are prone to damage (Osterud and Bjorklid, 2003). The oxidative modification of lipids and lipoproteins in LDL can ultimately result in the uptake of lipoproteins by arterial cells (Parthasarathy et al., 1999). This high concentration obviously has a chance to undergo LDL oxidation because it enters into the artery wall (Osterud and Bjorklid, 2003).

A previous study has showed that flavonoid fraction has antioxidant and antilipidperoxidative activity in HFD rats (Kaviarasan et al., 2008). Animals that received a flavonoid-rich fraction showed reduction in LDL-C and VLDL-C, evidencing the antihyperlipidemic nature of the flavonoid rich AIE. According to Ng et al., (1997) patients with IHDs experience markedly elevated triglycerides and reduced HDL-C levels. The authors attributed this to the metabolism of triglyceride-rich lipoproteins present in HDL-C, particularly the sub-fraction HDL2 which has a negative association with cardiovascular risk. Therefore, the increased serum triglycerides coupled with decreased HDL-C levels in ISO-treated rats as observed in this work, suggests predisposition to ischemic risk. HDL-C inhibits the uptake of LDL-C by the arterial wall and facilitates the transport of cholesterol from peripheral tissues to the liver where it is catabolised and excreted (Palanisamy and Kodukkur, 2010).

Flavonoids from AIE prophylactic and therapeutic treatment improved lipid profile with a significant increase in HDL levels and a decrease in total cholesterol, triglyceride, LDL and VLDL levels in myocardial ischemic rats, which corresponds to previous studies showing hypocholesterolaemic effect of flavonoids (DaSilva et al., 2001). This is an indication that the myocardial membrane is intact and not damaged.
Chapter-2

Plasma and Myocardial lipids

(Brewer, 1999; Sunmonu and Afolayan, 2010). Amlodipine owing to its lipid reducing property showed improvement in altered lipid profile of ISO-treated rats. In the present study, it was evident that ISO+AIE group was successfully able to negate the ISO induced perturbations in plasma lipid profile and elevate HDL level. Phospholipids, a vital component of biomembranes are important for the maintenance of cellular integrity, microviscosity and survival. Dietary fatty acids are also known to modulate in vivo lipid composition of biological membranes and consequently, their fluidity. The elevated plasma PL and FFA in ISO-treated rats may be due to increased peroxidation of membrane lipids via phospholipase A2 (Vijayapadma et al., 2006).

ISO rats showed elevated FFA that may be due to increased lipolysis in adipose tissue (Reddy et al., 2010). AIE pretreatment attenuated plasma FFA through its antilipolytic activity. Hesperetin (an aglycone of HDN) showed inhibition of epinephrine induced lipolysis, in a dose dependent manner (Kuppusamy and Das, 1992). It has been well documented that flavonoids such as quercetin, hesperetin and ursolic acid possessed hypolipidemic properties (Liu, 1995; Jeong et al., 2003; Juzwiak et al., 2005). Flavonoid-rich AIE pretreatment reduced plasma FFA and PL concentrations in myocardial ischemic rats showing that the active ingredients present in flavonoid-rich fraction might be due to the free radical scavenging property (Malterud and Rydland, 2000) and thus play a role in maintaining membrane integrity and activities of membrane-bound enzymes.

Myocardial lipids

Lipid metabolism plays an important role in myocardial necrosis produced by ischemia (Mathew et al., 1981). An ISO-treated rat showing altered lipid profiles in the heart agrees well with a previous report (Senthil et al., 2007). The significant increase observed in the lipid profiles except phospholipids in the rat treated with ISO alone could be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate (cAMP) (Paritha and Devi, 1997). A significant rise in cholesterol content suggested that the redistribution of cholesterol in ischemic cells. The elevated level of cholesterol in heart is well associated with myocardial ischemia (Venter et al., 1991). The significant rise in heart TG in ISO induced rats might be due to decrease in the activity of lipoprotein lipase, resulting in decreased uptake of TGs from the circulation (Prince and Rajadurai, 2005). The mechanism of observed increase in the
synthesis of TGs in the heart tissue could also be due to accumulation of acyl-CoA and an augmented production of glycerol by increased glycolytic flux (Subramanian et al., 2003). A significant decrease in phospholipid content could be due to an accelerated degradation of membrane phospholipids by phospholipases (Palanisammy and Kodukkur, 2010). We observed an increase in the level of FFAs in the heart of ISO-induced rats. In aerobic conditions, cardiomyocytes prefer free fatty acids for energy. Ischemic myocardium is not in a position to oxidize the available fatty acids and results in the accumulation of fatty acyl coA derivatives. The heart derives a significant portion of its fatty acid substrates as FFAs derived by lipolysis from adipose tissue. Although lipid availability is important for the heart, excess levels of fatty acid in myocytes can be deleterious.

Prior treatment with flavonoid rich AIE lowered the levels of FFAs and increased PLs in myocardial ischemic rats compared to synthetic drug, amlodipine treated groups. In this study, AIE treatment was shown to attenuate the alterations of myocardial lipids. This may be due to its anti-lipid peroxidative effect on cardiomyocytes.
### Table 2.1: Effect of flavonoid rich AIE on plasma lipids 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>TC %</td>
<td>74.75 ± 6.14</td>
<td>73.35 ± 5.64</td>
<td>107.58 ± 9.19</td>
<td>85.73 ± 5.85</td>
<td>92.56 ± 6.46</td>
</tr>
<tr>
<td>TG %</td>
<td>52.78 ± 3.27</td>
<td>51.36 ± 3.09</td>
<td>72.93 ± 5.77</td>
<td>53.81 ± 2.91</td>
<td>59.58 ± 3.23</td>
</tr>
<tr>
<td>HDL-C %</td>
<td>42.78 ± 3.24</td>
<td>42.13 ± 3.11</td>
<td>30.78 ± 2.26</td>
<td>43.48 ± 2.53</td>
<td>45.16 ± 3.05</td>
</tr>
<tr>
<td>VLDL-C %</td>
<td>10.55 ± 0.65</td>
<td>10.27 ± 0.61</td>
<td>14.58 ± 1.15</td>
<td>10.76 ± 0.58</td>
<td>11.91 ± 0.64</td>
</tr>
<tr>
<td>LDL-C %</td>
<td>21.41 ± 8.31</td>
<td>20.94 ± 5.85</td>
<td>62.21 ± 7.05</td>
<td>31.48 ± 6.49</td>
<td>35.48 ± 4.81</td>
</tr>
<tr>
<td>PL %</td>
<td>80.35 ± 6.22</td>
<td>79.28 ± 6.04</td>
<td>119.61 ± 10.68</td>
<td>81.04 ± 5.14</td>
<td>88.86 ± 7.87</td>
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<tr>
<td>FFA %</td>
<td>50.18 ± 3.56</td>
<td>49.58 ± 3.23</td>
<td>68.81 ± 6.39</td>
<td>53.48 ± 3.44</td>
<td>59.13 ± 3.91</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. of 6 individual rats. Values in the parenthesis are % change (a compared to ‘CON’, b compared to ‘ISO’). Values not sharing a common super script differ significantly at cP < 0.05, dP < 0.05, eP < 0.05 vs control (DMRT).
Table 2.2: Effect of flavonoid rich AIE on myocardial lipids in the control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>CON+AIE</td>
<td>ISO</td>
<td>ISO+AIE</td>
<td>ISO+AML</td>
</tr>
<tr>
<td>TC %</td>
<td>3.56 ± 0.12</td>
<td>3.39 ± 0.14</td>
<td>4.94 ± 0.10</td>
<td>3.82 ± 0.11</td>
<td>4.21 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>(4.77)h</td>
<td>(38.76)h</td>
<td>(-22.67)*</td>
<td>(-14.77)*</td>
<td></td>
</tr>
<tr>
<td>TG %</td>
<td>3.74 ± 0.11</td>
<td>3.52 ± 0.13</td>
<td>5.49 ± 0.18</td>
<td>4.36 ± 0.12</td>
<td>4.61 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>(-5.88)h</td>
<td>(46.79)h</td>
<td>(-20.58)*</td>
<td>(-16.02)*</td>
<td></td>
</tr>
<tr>
<td>PL %</td>
<td>13.04 ± 1.41</td>
<td>12.92 ± 1.04</td>
<td>9.31 ± 0.92</td>
<td>12.26 ± 1.05</td>
<td>11.83 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>(-0.92)h</td>
<td>(-28.60)h</td>
<td>(31.68)*</td>
<td>(27.06)*</td>
<td></td>
</tr>
<tr>
<td>FFA %</td>
<td>3.95 ± 0.16</td>
<td>3.83 ± 0.10</td>
<td>5.02 ± 0.20</td>
<td>4.11 ± 0.18</td>
<td>4.52 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>(-3.03)h</td>
<td>(27.08)h</td>
<td>(-18.12)*</td>
<td>(-9.96)*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D. of 6 individual rats.
Values in the parenthesis are % change (' compared to ‘CON’, * compared to ‘ISO’).
Values not sharing a common super script differ significantly at 'P < 0.05, bP < 0.05, cP < 0.05, dP < 0.05, eP < 0.05 vs control (DMRT).
Fig 2.1: Effect of flavonoid rich AIE on plasma total cholesterol 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, bP < 0.05, cP < 0.05 vs control (DMRT).

Fig 2.1.1: % change of plasma total cholesterol levels in control and experimental animals

Values are % change (#compared to ‘CON’, *compared to ‘ISO’).
Fig 2.2: Effect of flavonoid rich AIE on plasma triglycerides 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, ‡P < 0.05 vs control (DMRT).

Fig 2.2.1: % change of plasma triglycerides levels in control and experimental animals

Values are % change (*compared to 'CON', #compared to 'ISO')
Fig 2.3: Effect of flavonoid rich AIE on plasma HDL-C in control and experimental animals

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

Fig 2.3.1: % change of plasma HDL-C levels in control and experimental animals

Values are % change (\(^a\) compared to 'CON', \(^*\) compared to 'ISO')
Fig 2.4: Effect of flavonoid rich AIE on plasma VLDL-C 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 2.4.1: % change of plasma VLDL-C levels in control and experimental animals

Values are % change ('compared to 'CON', 'compared to 'ISO')
Fig 2.5: Effect of flavonoid rich AIE on plasma LDL-C in control and experimental animals

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

Fig 2.5.1: % change of plasma LDL-C levels in control and experimental animals

Values are % change (\(^#\) compared to ‘CON’, \(*\) compared to ‘ISO’)

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Fig 2.6: Effect of flavonoid rich AIE on plasma phospholipids 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 2.6.1: % change of plasma phospholipids in control and experimental animals

Values are % change ("compared to ‘CON’, * compared to ‘ISO’)
Fig 2.7: Effect of flavonoid rich AIE on plasma free fatty acids 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: 2.7.1: % change of plasma free fatty acids in control and experimental animals

Values are % change ("compared to 'CON', *compared to 'ISO'")
Fig 2.8: Effect of flavonoid rich AIE on myocardial total cholesterol in control and experimental animals

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

Fig 2.8.1: % change of myocardial total cholesterol levels in control and experimental animals

Values are % change (\(^*\)compared to ‘CON’, \(^*\)compared to ‘ISO’)

115
**Fig 2.9**: Effect of flavonoid rich AIE on myocardial triglycerides in control and experimental animals

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

**Fig 2.9.1**: % change of myocardial triglycerides levels in control and experimental animals

Values are % change ($^#$compared to ‘CON’, *compared to ‘ISO’)

116
Fig 2.10: Effect of flavonoid rich AIE on myocardial phospholipids 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, \( ^{b}P < 0.05 \) vs control (DMRT).

Fig 2.10.1: % change of myocardial phospholipids levels in control and experimental animals

Values are % change (*compared to ‘CON’, *compared to ‘ISO’).
Fig 2.11: Effect of flavonoid rich AIE on myocardial free fatty acids 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 2.11.1: % change of myocardial free fatty acids levels in control and experimental animals

Values are % change ("compared to 'CON", *compared to 'ISO")