Results

Blood glucose levels were measured using glucometer (Accu Chek) in all groups during the experimental period. Blood glucose concentration was not changed in AIE alone treated rats, whereas ISO treatment induced significantly increased concentration. With the amlodipine pretreatment to ISO induced ischemic rats, blood glucose concentrations decreased at 21.37%. Interestingly, flavonoids rich AIE significantly decreased the blood glucose by 27.35% (P<0.05 compared with ISO alone), suggesting antihyperglycemic activity of AIE.

Rats injected with ISO for two consecutive days showed a significant increase in body weight, heart weight and heart to body weight ratio as compared to controls. Treatment with flavonoid rich AIE alone to normal rats did not show significant alterations, in contrast pretreatment with AIE in ISO injected rats showed a significant improvement in body weight, heart weight and heart to body weight ratio as compared to ISO injected and amlodipine alone pretreated ISO injected rats.

Discussion

There is direct evidence that high blood glucose can cause cardiovascular disease. High levels of blood glucose are known to cause damage to the arteries and blood vessels. Blood vessels and arteries are dynamic and they are in a constant state of change and adapt to the demands that are put upon. The changes that occur are controlled by endothelial cells. These cells line inside wall of the blood vessels and arteries and
perform a wide range of different tasks. They generally keep everything within vessels and arteries balanced and under control. The presence of too much glucose in the blood can actually impair the normal functioning of the endothelial cells (Webster and Scott, 1997; Ludwig, 2002). In particular, high blood glucose can prevent blood vessels from dilating (widening). This, of course, has very important implications for the flow of blood and oxygen through the blood vessels to the heart. Endothelial cells synthesize a substance called nitric oxide (NO) that actually causes blood vessels to widen so that blood can flow through more easily (Beckman et al., 2002).

High levels of blood glucose can actually inhibit the ability of endothelial cells to manufacture nitric oxide (Title et al., 2000). Furthermore, nitric oxide is also involved in a number of other functions that protect against heart disease (Beckman et al., 2002). Overall, problems with the functioning of endothelial cells form a key step in the progression of ischemic heart disease (Title et al., 2000). According to the American Diabetes Association (2005) normal blood glucose level is less than 100.9 mg/dL (fasting glucose) and 109.9 mg/dL (2-h values in the oral glucose tolerance test (OGTT)). Hyperglycemia may harm myocardium through multiple pathways. It leads to increased production of reactive oxygen species (ROS) through the hexosamine biosynthetic pathway induced by isoproterenol (Haber et al., 2003, Kim et al., 2006). In cultured ventricular myocytes incubated in a medium containing high concentrations of glucose, free radical generation (especially ROS production) and proinflammatory cytokine concentrations were drastically raised, and the number of dead and apoptotic myocytes markedly increased (Fiordaliso et al., 2004).

Therefore, it is likely that the increased oxidative stress induced by hyperglycemia may be the primary mechanism responsible for enhanced ischemia injury observed in animals treated with high glucose. Furthermore, using a human ventricular heart cell model of simulated ischemia, Verma et al., (2002) demonstrated that cellular injury was greater in human ventricular heart cells subjected to hyperglycemic conditions. Hyperglycemia represents increased blood glucose level that is result of activation of neurohormonal processes in organism exposed to stress induced by ISO (Hui et al., 2007;
Although the stress response enables the organism under attack to prepare itself quickly for fighting or escaping, prolonged or repeated stress is associated with a variety of disorders, including cardiovascular diseases such as myocardial ischemia (Nonogaki, 2000). Increased glucose level during stress is result of sympathetic nervous system activation and catecholamines (isoproterenol) that stimulate processes of glyconeogenesis, glycogenolysis and lipolysis. These are responsible for insulin resistance, on receptor and post receptor level, so there are in the same time hyperglycemia, hyperinsulinemia and insulin resistance (Goran et al., 2006).

Cardiac tissue and adipocytes are supersensitive to isoproterenol and subsensitive to noradrenaline (Vanderlei et al., 1996; Farias et al., 1999). Verago et al., (2001) examined the effect of an infusion of ISO on plasma glucose in rats. In our study blood glucose levels were maintained at normal levels in control rats and rats treated with flavonoid rich AIE alone. Blood glucose levels were increased significantly in ISO alone treated rats when compared to normal rats. Flavonoid rich AIE pretreated rats administered with ISO showed significant decrease (P<0.05) in blood glucose when compared to rats given ISO alone and rats pretreated with amlodipine. This observation is in accordance with the earlier report of Leigh et al., (2000) may be due to the insulin like biological activity of AIE. The possible mechanism for the observed anti-hyperglycemic effect of flavonoid rich AIE treatment may be due to increased ability of insulin to mediate tissue glucose uptake and thus helpful to maintain glucose homeostasis.

A good number of studies have already demonstrated the hypoglycemic effects of flavonoids using different experimental models and treatment has been shown to exert such beneficial effects against the disease manifestation, either through their capacity to avoid glucose absorption or to improve glucose tolerance. It has also been demonstrated that flavonoids can act per se as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the disease complications; besides, they have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. As a result, bio-flavonoids are now-a-days regarded as promising
and significantly attractive natural substances to enrich the current therapy options against hyperglycemia (Brahmachari, 2011). Choi et al., (1991) demonstrated that intraperitoneal administration of prunin (naringenin 7-O-β-D-glucoside) produces a significant hypoglycemic effect in diabetic rats. Anti-hyperglycemic effects have also been demonstrated for various flavonoids including chrysin and its derivatives, silymarin, isoquercetrin and rutin (Velussi et al., 1997; Shin et al., 1999; Hnatyszyn et al., 2002). Synthetic derivatives of flavonoids have been shown to release insulin from insulinotropic INS-1 cells in culture, which further supports our finding that the flavonoid-rich extract probably acts as an insulinotrophic agent as well (Bozdag et al., 2001).

Heart weight/body weight (HW/BW) ratio measurements are generally used for assessment of the hypertrophic index. Alterations in HW/BW ratio could be arise due to consecutive loss of myocardial connective tissue in damage myocardium. In the present study, we noted that there was a significant HW/BW ratio increment in ISO-induced rats but concurrent treatment with flavonoid rich AIE markedly inhibited ISO-induced HW/BW ratio increment suggestive of its myocardial connective tissue protective effects. Significant increase in the heart weight of ISO-treated rats has been attributed to increased water content, formation of oedematous intramuscular spaces, extensive necrosis of cardiac muscle fibres and invasion by inflammatory cells (Thounaojam et al., 2011). In the current study, pre-supplementation with AIE could significantly minimise increase in heart weight possibly due to prevention of edema (Devika and Mainzen, 2008).

ISO treatment also produced cardiac hypertrophy as reflected by increased heart weight to body weight, which is in agreement with earlier report (Bhulan et al., 2011). Hypertrophy is believed to be due to increase in water content and development of oedema in intramuscular spaces culminating in extensive necrotic changes and invasion of inflammatory cells (Nirmala and Puvanakrishnan, 1996). Amlodipine treatment improved hypertrophy owing to its cardioprotective properties as reported in earlier study (Bhulan et al., 2011). The expression of subunits (Fukui et al., 2001) and the enzyme
activity (Heymes et al., 2003) of NADPH oxidase has been shown to increase in ischemic myocardium and may contribute to cardiac hypertrophy (Looi et al., 2008). Although not yet investigated for this mechanism in ischemia, flavonoids have shown ability to suppress enzyme activity and/or expression of NADPH oxidases (Masoumeh and Brian, 2009). In our study pretreatment with flavonoid rich AIE prophylactically as well as therapeutically improved cardiac performance and reduced the heart weight to body weight ratio (hypertrophic index), reflecting a cardioprotective effect.
Table 1.1: Effect of flavonoid rich AIE on blood glucose and hypertrophic index 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>Blood glucose %</td>
<td>80.04±2.93</td>
<td>80.31±3.23</td>
<td>111.73±3.73</td>
<td>81.17±2.26</td>
<td>87.85±3.52</td>
</tr>
<tr>
<td></td>
<td>(0.33)</td>
<td>(0.33)</td>
<td>(39.59)</td>
<td>(-27.35)</td>
<td>(-21.37)</td>
</tr>
<tr>
<td>Heart weight (HW) %</td>
<td>0.686±0.20</td>
<td>0.708±0.19</td>
<td>1.194±0.40</td>
<td>0.864±0.30</td>
<td>0.975±0.49</td>
</tr>
<tr>
<td></td>
<td>(3.20)</td>
<td>(3.20)</td>
<td>(74.05)</td>
<td>(-27.63)</td>
<td>(-18.34)</td>
</tr>
<tr>
<td>Body weight (BW) %</td>
<td>210.51±8.13</td>
<td>212.19±6.30</td>
<td>241.44±9.82</td>
<td>200.94±6.31</td>
<td>221.14±7.71</td>
</tr>
<tr>
<td></td>
<td>(0.79)</td>
<td>(0.79)</td>
<td>(14.69)</td>
<td>(-16.77)</td>
<td>(-8.40)</td>
</tr>
<tr>
<td>HW/BW %</td>
<td>3.25±0.15</td>
<td>3.33±0.10</td>
<td>5.16±0.26</td>
<td>3.69±0.22</td>
<td>4.07±0.20</td>
</tr>
<tr>
<td></td>
<td>(2.14)</td>
<td>(2.14)</td>
<td>(58.2)</td>
<td>(-28.48)</td>
<td>(-21.12)</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 \(^aP < 0.05\), \(^bP < 0.05\), \(^cP < 0.05\), \(^dP < 0.05\) vs control (DMRT).
**Fig 1.1:** Effect of flavonoid rich AIE on blood glucose in control and experimental animals

![Graph showing blood glucose levels](image)

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 1.1.1:** % change of blood glucose levels in control and experimental animals

![Graph showing % change in blood glucose levels](image)

Values are % change ('compared to 'CON', * compared to 'ISO')
**Fig 1.2:** Effect of flavonoid rich AIE on heart weight in control and experimental animals

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

**Fig 1.2.1:** % change of heart weight in control and experimental animals

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

98
Fig 1.3: Effect of flavonoid rich AIE on body weight in control and experimental animals

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

Fig 1.3.1: % change of body weight in control and experimental animals

Values are % change (\(^{a}\) compared to 'CON', \(^{c}\) compared to 'ISO')
Fig 1.4: Effect of flavonoid rich AIE on heart weight/body weight ratio in control and experimental animals

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

Fig 1.4.1: % change of heart weight/body weight ratio in control and experimental animals

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