Cardioprotective study of *C. ternatea* and *Aloe vera* gel
INTRODUCTION

Cardiovascular disease (CVD) remains the principal cause of death in both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year. Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (De Bono and Boon, 1992; Karthik and Prince, 2006), due to that there is an imbalance formed between oxygen supply and demand.

Isoproterenol (ISO), a β-adrenergic agonist, has been found to cause severe stress in myocardium, resulting in infarct-like necrosis of heart muscle with an increase in the level of lipids in the myocardium (Wexler and Greenberg, 1978). Free radical generation and lipid peroxidation are involved in ISO-induced cardiac damage and pathophysiological changes following ISO administration are similar to those taking place in human MI (Sangeetha and Quine, 2006).

Dose dependent cardiotoxicity is the most important adverse effect of prolonged (24h) β-agonist administration in animals, resulting in histological changes (Rona et al., 1959). This is reported that death occurred in animal with low dose (21.2 mg/kg) of ISO administrations are mainly due to severe heart lesions. The animal which died at doses of 170mg/kg or greater also revealed liver and kidney necrosis, hydrothorax and hemorrhagic lung edema. Based on previous data we have selected the dose 5.25 mg/kg and 8.5 mg/kg for two subsequent days to produce myocardial infarction in rats (Rona et al., 1959). A number of substances have been identified for their ability to protect against experimental myocardial infarction induced by isoproterenol (Wexler and Greenberg, 1978). Any substance that can prevent an attack or accelerate the process of recovery will have considerable clinical application.
Various studies have been done to test compounds against various doses of ISO induced MI. Karthick and Prince (2006), reported protective effect of rutin at dose of 40 mg/kg for 42 days followed by ISO (150 mg/kg, once a day for two days). They reported protection is due to decreased activity of cardiac marker enzyme viz. creatine kinase, LDH, AST and ALT in serum of rutin pretreated animal serum. They also found reduction in plasma and myocardial TBARS and lipid hydroperoxides level in toxic group pretreated with rutin. Sngeetha and Quine reported that alliiin (s-ally cysteine sulfoxide) also give protection by controlling the levels of marker enzymes of MI along with serum LDL, and VLDL and HDL cholesterol level. Alliiin also maintains free fatty acids and phospholipids at near-normal level. It is reported that the increase in level of free fatty acids in serum in ISO treated animals is due to increased lipolysis (Saleena et al., 1981). The decreased level of free fatty acids in serum at peak infraction in animals pretreated with carnitine is due to decreased lipolysis, increased uptake by mitochondria or both (Kumari and Menon, 1988).

Traditional therapies for ischemia are aimed at restoring the balance between oxygen delivery and the myocardial demand of oxygen (Mitra and Panja, 2005). Ayurvedic formulation Abana also showed protective effect by controlling marker enzymes level along with lipid peroxidation in serum and heart and glutathione level in blood and heart tissue (Sashikumar and Devi, 2000). Mohanty et al. (2004), reported that ISO treatment at doe of 85 mg/kg for two consecutive days showed a significant decrease in GSH, activities of superoxide dismutase, catalase, creatinine phosphokinase and lactate dehydrogenase as well as increase in lipid peroxidation levels of heart. However, they did not observe any significant change in activity of glutathione peroxidase and protein levels. Left ventrical dysfunction was seen as a decrease in heart rate, Withenia somnifera exert a protective effect against ISO induced cardiotoxicity. In view of all these data we have
evaluated the cardioprotective efficiency of aqueous extract of Clitoria ternatea and 30% Aloe vera gel against ISO induced myocardial infraction (MI).

MATERIAL AND METHODS

Drug schedule

Isoproterenol was procured from Sigma chemicals (St Louis, MO, USA), and dissolved in normal saline. Dried aqueous extract of Clitoria ternatea and Aloe vera gel was resuspended in normal saline for making solutions and given orally for the period of 20 days. The dose of C. ternatea (100 mg/kg) and Aloe vera gel (200 mg/kg) was selected on the basis of their potential for antidiabetic activity based on our study. Isoproterenol was given subcutaneously at dose 5.25 mg/kg and 8.5 mg/kg for two subsequent days on 19th and 20th day. All the rats were sacrificed on 21st day for various biochemical estimations.

Animals

Animals were divided in to five groups consisting of six animals in each group-

I Rats given normal saline (1 ml/kg, p.o.) once daily.

II normal saline (1 ml/kg, p.o.) once daily + Isoproterenol (s.c., 5.25 and 8.5 mg/kg).

III 30% Aloe vera gel (200mg/kg, p.o.) + Isoproterenol (s.c 5.25 and 8.5 mg/kg).

IV Aqueous extract of C. ternatea (100 mg/kg, p.o.) + Isoproterenol (s.c. 5.25 and 8.5 mg/kg).

V Gliclazide (25 mg/kg, p.o.) + Isoproterenol (s.c.5.25 and 8.5 mg/kg).
Blood glucose, Glycosylated Haemoglobin (HbA1c), Serum lactate dehydrogenase, Serum creatine kinase was evaluated by using various kits.

For blood GSH fresh blood was diluted with distilled water and filtrate was collected by addition of precipitating solution. The 2.0 ml of filtrate was added to 8.0 ml of phosphate solution (Beutler et al., 1963). The OD was measured at 412 nm after addition of DTNB. Lipid peroxidation was studied out by measuring the formation of thiobarbituric acid reactive substance according to method of Okhawa (1979).

Tissue GSH was estimated by method of sedlekk and Lindsay (1968) by using Ellman reagent.

For catalase estimation tissues were homogenized in PBS, supernatant was added to 19mM solution of H$_2$O$_2$. Disappearance of H$_2$O$_2$ was monitored at 240 nm for 3 min (Clairborne et al., 1985). SOD estimation was done by method of Marklund (1985), in which cytosolic supernatant was added to tris buffer (pH 8.5) at last pyrogallol was added and inhibition of pyrogallol was observed in presence of SOD at 420nm.

Protein was estimated by Lwory's method (Lwory et al., 1959) with Folin's Ciocalteau phenol reagent.

For histopathological examination, tissues were fixed in 10% natural buffered formaline solution. After that tissue was processed for dehydration, clearing with chloroform, impregnation in paraffin wax, then sections of 5 μ in thickness were cut with the help of rotary microtome, out of 10 sections 3 sections were evaluated for histological findings.
RESULTS AND OBSERVATIONS

Present investigation was done to evaluate the activity of the effective doses of aqueous extract of *Clitoria tematea (CT)* and 30% *Aloe Vera Gel (AG)* on oxidative stress in myocardial infraction. Myocardial infraction was induced by two subsequent s.c. injection of isoproterenol (ISO). Serial investigations were performed in all the groups for the estimations of heart rate and blood pressure, blood glucose, blood glutathione, glycosylated hemoglobin, serum lactate dehydrogenase (LDH), serum creatine kinase (CK), tissue lipid peroxide (TBARS), tissue glutathione (GSH), tissue superoxide dismutase (SOD) & tissue catalase levels.

1. **Haemodynamic parameters**

   The heart rate of control group was 395.0± 2.2 beats/min. conversely, in the isoproterenol-treated rats, there was a significant rise in the heart rate, as compared to normal rats. However, administration of aq. CT and 30 % *Aloe vera* gel significantly reduced heart rate when compared to pathogenic myopathic rats. Gliclazide also showed reduction in heart rate in isoprenaline treated rats (Figure 6.1).

   The mean systolic BP of normal rats was found to be 137.0 ± 6.5 mmHg. Isoproterenol administration significantly reduces the mean blood pressure. 30 % *Aloe vera gel* and *C. ternatea* treatment significantly maintained the mean BP levels as compared to pathogenic animals. Gliclazide treatment did not show any significant recovery of mean blood pressure blood pressure when compared to pathogenic rats (Figure 6.1).

2. **Biochemical studies**

   2.1 Biochemical studies in blood

   Table 6.1 shows the effect of drug treatment on blood glucose, HbA1c and blood GSH level in isoproterenol treated rats. The mean blood
glucose level in rats healthy control group alone was stable (69.3±0.7 mg/dl) throughout the experimental period. Conversely, in isoproterenol treated groups (i.e. pathogenic control group) there was a significant raise in the blood glucose level, as compared to healthy animals. Pretreatment with *Clitoria ternatea* and *Aloe vera gel* in ISO treated rats for 20 days, significantly reduced blood glucose levels compared to pathogenic rats. Gliclazide (25 mg/kg/day, p.o.) pretreatment also maintained the blood glucose level when compared to pathogenic control group. The glycated Hb level in normal healthy rats was found to be 5.05±0.2% whereas in pathogenic rats, there was no significant increase in HbA₁c levels as compared to control rats. *C. ternatea and Aloe vera gel* (CT-100 mg/kg and AG- 200 mg/kg) treatment for 20 days also did not alter the level of HbA₁c respectively as compared to pathogenic rats. Gliclazide also could not produce any effect on the HbA₁c levels as compared to pathogenic control group. The blood GSH level in normal control group rats was found to be 3.36±0.07 mg/dl. ISO treatment in rats resulted in more than 60% decrease in blood GSH levels as compared to normal healthy rats. *C. ternatea* treatment (100 mg/kg/day) and *Aloe vera gel treatment* (200 mg/kg/day) for 20 days significantly restore the blood GSH levels as compared to pathogenic animals. Gliclazide treatment in myopathic rats also showed significant increase in blood GSH levels when compared to pathogenic control rats.

2.2 **Biochemical studies in serum**

The serum LDH levels in normal control animals (group I) were found to be 152.2±2.4 IU/L whereas in rats treated with two subsequent s.c. injection of isoproterenol showed significant increase in serum LDH levels, compared to normal healthy control rats. However, pretreatment with aqueous extract of CT (100 mg/kg) and 30% AG (200 mg/kg) for 20
days before ISO injection in rats significantly reduced the serum LDH levels as compared to pathogenic control rats (Fig 6.2).

The serum CK levels in normal control animals were found to be 82.2±1.4 lU/L whereas in rats treated with ISO only (pathogenic control group) showed about five folds increase in serum CK levels as compared to healthy rats. However, test drug treatment (CT, 100 mg/kg, p.o. and 30% AG, 200 mg/kg, p.o.) in rats for 20 days significantly reduced the serum CK levels as compared to pathogenic control rats. Gliclazide treatment also showed a significant reduction in serum CK level (Fig 6.2).

2.3 Biochemical studies in tissues (heart, liver and pancreas)

Table 6.2 represents the effect of various drugs on TBARS level. The lipid peroxide levels in the heart homogenate of normal control rats were found to be 0.53±0.04 nmoles MDA/mg protein. ISO treatment in rats raised the levels of TBARS in heart tissue by about 7-folds (pathogenic rats) as compared to normal control rats. C. ternatea extract and Aloe vera gel pretreatment significantly reduce the levels of lipid peroxides as compared to pathogenic rats group. Gliclazide treatment do not showed significant reduction in lipid peroxide levels in ISO-treated rats on 20 days pretreatment. The lipid peroxide levels in the liver of normal control rats (group I) were found to be 0.47±0.03 nmoles MDA/mg protein. ISO- treated rats showed significant rise lipid peroxide levels as compared to healthy rats. C. ternatea (100 mg/kg) and 30% AG (200 mg/kg) significantly reduced the level of lipid peroxides as compared to pathogenic rats. Gliclazide treated rats do not showed significant reduction in lipid peroxide levels as compare to pathogenic rats. The lipid peroxide levels in pancreas of normal healthy control rats (group I) were found to be 0.47±0.04 nmoles MDA/mg protein. Pathogenic cardiomyopahtic rats showed significantly higher lipid peroxide levels as compared to normal healthy rats. C. ternatea extract and Aloe vera gel treatment (100 mg/kg and 200 mg/kg
respectively) significantly reduced lipid peroxide levels as compared to pathogenic rats. The gliclazide pretreatment also produced significant reduction in lipid peroxide levels compared to isoproterenol treated rats.

Table 6.3 shows the effect of various treatments on tissue GSH level. The mean levels of glutathione in heart of control rats were found to be 99.6±6.0 µg/mg protein. ISO treatment in pathogenic rats significantly decreased the GSH level as compared to normal healthy control rats. Drug pretreatment (100 mg/kg of CT aqueous extract & 200 mg/kg of 30% AG) in ISO treated rats for 20 days showed significant recovery of GSH as compared to pathogenic rats. Gliclazide treatment showed significant increase in GSH level as compared to pathogenic rats. There was statistically significant decrease in the liver GSH levels of pathogenic ISO treated rats as compared to normal healthy control rat's liver (68.3±2.2pg/mg protein). Pretreatment with both the drugs for 20 days in ISO-treated rats showed significantly increased glutathione levels at dose of 100 mg/kg with CT and 200 mg/kg of 30% AG. Furthermore, Gliclazide do not showed significant increase in GSH level in liver on 20 days pretreatment as compared to pathogenic rats. The mean levels of glutathione in pancreas of control rats were found to be 45.2± 2.9 µg/mg protein. ISO treatment in rats significantly decreased the GSH level as compared to healthy rats. Drug pretreatment (100 mg/kg of aqueous extract of CT and 200 mg/kg of 30% AG) in ISO treated rats for 20 days showed significant recovery of GSH as compared to pathogenic rats. Gliclazide pretreatment also showed significant increase in GSH level as compared to pathogenic rats.

Table 6.4 represents the effect of various treatments on tissue catalase level. The mean catalase level in heart of rat fed a normal diet was found to be 2.4±0.1 nmoles H₂O₂/min/mg protein. Conversely, in the isoproterenol-treated rats group, there was a significant fall in heart catalase
levels as compared to healthy animals. Both C. ternate aqueous extract and 30% AG significantly increased the level of catalase in isoproterenol treated rats as compared to pathogenic rats. Pretreatment with gliclazide for 20 days do not showed significant recovery in catalase levels compared to pathogenic rats. The mean catalase level in liver of healthy control group rat was found to be 2.93±0.6 nmoles H$_2$O$_2$/min/mg protein. Conversely, in the isoproterenol-treated rats group, there was a significant fall in liver catalase levels as compared to group normal healthy rats. C. ternatea (100 mg/kg) and 30% AG (200 mg/kg) significantly maintained the level of catalase as compared to pathogenic rats and tend it towards normal. Gliclazide pretreated rats for 20 days do not showed significant increase in catalase levels in comparison to pathogenic rats. The mean catalase level in pancreas of healthy control group was found to be 0.98±0.01 nmoles H$_2$O$_2$/min/mg protein. Conversely, in the isoproterenol-treated rats group, there was a significant reduction in pancreatic catalase levels as compared to normal healthy rats. Pretreatment with aqueous extract of C. ternatea (100 mg/kg) and 30% AG (200 mg/kg) significantly increases the level of catalase as compared to pathogenic rats. Administration of gliclazide for 20 days before ISO administration also showed significant increase in catalase levels compared to pathogenic rats.

Table 6.5 shows the effect of various treatments on tissue SOD levels. The mean SOD levels in heart supernatant fell in pathogenic ISO treated rats was significant as compared to normal healthy (1.61±0.03 IU/mg protein). Test drug, C. ternatea (100 mg/kg) and Aloe vera gel (200 mg/kg), significantly increase SOD levels as compared to pathogenic group rats. Gliclazide pretreatment also exhibit significantly higher SOD levels when compared to pathogenic rats. Isoproterenol treatment significantly decreased superoxide dismutase levels in liver when compared to normal healthy rats (1.73±0.13 IU/mg protein). Aqueous extract of C. ternatea (100
mg/kg) and 30% Aloe Vera Gel (AG) at dose of 200 mg/kg showed a significant increase in liver SOD levels when compared to pathogenic rats. Gliclazide pretreatment in ISO treated rats also showed significant increase in SOD level as compared to toxic group.

The mean SOD levels in Pancreatic supernatant fell significantly in pathogenic ISO treated diabetic rats when compared to normal healthy (group 0.88±0.04 IU/mg protein). Test drug pretreatment i.e. C. ternatea (100 mg/kg) and 30% AG (200 mg/kg) significantly increase SOD levels as compared to pathogenic group rats. Gliclazide (25 mg/kg) pretreated rats on administration of ISO also exhibit significantly higher SOD levels when compared to pathogenic rats.

3. Anthropometrics measurement

No significant change in food and water intake was observed in diabetic and drug treated rats.

4. Histophathological observations

Figure 6.3 shows the histopathological photographs of heart section of various groups. Heart section of normal healthy control rats showed normal cardiac muscle bundles, normal myocardium with striations, branched appearance and continuity with adjacent myofibrils. While ISO treated rats' heart showed edema, focal haemorrhage with congestion and leukocyte infiltrate. Rats pretreated with extract of C. ternatea at dose of 100 mg/kg showed less congestion and partial leukocyte adhesion with normal myocardium. 30% AG at dose of 200 mg/kg pretreated rats which were then treated with sc injection of ISO for two subsequent days showed normal myocardium with striations these findings are comparable to normal rat heart section. Gliclazide pretreated rats on ISO administration showed myocardium with petechial hemorrhage.
Figure 6.4 shows the histopathological photographs of liver section of various groups. Histopathological section of normal healthy control rat liver showed normal hepatocytes arranged in chords with portal triads and central viens and normal sinusoid while ISO treated rat's liver showed marked congestion in blood vessels and sinusoids. Rats pretreated with 100 mg/kg of aqueous extract of CT showed mild feathery degeneration of hepatocytes and pretreatment with 30%AG at dose of 200 mg/kg showed very mild cellular degeneration with normal portal triads. Gliclazide pretreatment in ISO treated rat's showed feathery degeneration of hepatocytes in centrilobular area.

Figure 6.5 shows the histopathological photographs of pancreatic section of various groups. Pancreatic section of normal healthy rat showed globules of acini with islet cells, while isoprpterenol treated rat's pancreas showed congestion with less number of islet cells. Pretreatment with aqueous extract of C. ternatea (100 mg/kg). ISO treated rat with pretreatment of showed feathery degeneration, AG- 200 mg/kg showed globules of pancreatic acini with groups of islet cells. Gliclazide pretreated rat pancreatic section showed few necrotic B-cell in islet with degeneration.
Figure 6.1: Effect of pretreatment of 30% Aloe vera gel and aqueous extract of Clitoria ternatea on mean blood pressure, heart rate and heart rate in isoproterenol treated rats

\[ \text{CON} \quad \text{ISO} \quad \text{ISO+AG} \quad \text{ISO+CT} \quad \text{ISO+GLI} \]

- MBP (mmHg)
- Heart rate (beats/min)

\[ ^a P < 0.01, \text{ as compared to group I (ANOVA followed by Dunnett's t test)} \]
\[ ^b P < 0.01, \text{ as compared to group II (ANOVA followed by Dunnett's t test)} \]
\[ ^c P < 0.05, \text{ as compared to group II (ANOVA followed by Dunnett's t test)} \]
Figure 6.2: Effect of pretreatment of 30% Aloe vera gel and aqueous extract of Clitoria ternatea on serum lactate dehydrogenase (LDH) and creatine kinase (CK) levels in isoproterenol treated rats

- □ CON
- ■ ISO
- □ ISO+AG
- □ ISO+CT
- ■ ISO+GLI

- • $P<0.01$, as compared to group I (ANOVA followed by Dunnett’s t test)
- • $P<0.01$, as compared to group II (ANOVA followed by Dunnett’s t test)
- • $P<0.05$, as compared to group II (ANOVA followed by Dunnett’s t test)
Table 6.1: Effect of aqueous extract of Clitoria Ternatea (CT-100 mg/kg) and 30% Aloe Vera Gel (AG-200 mg/kg) pretreatment on blood glucose, blood glycosylated haemoglobin (HbA1c), blood glutathione (GSH), levels in rats treated with isoproterenol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Blood Glucose (mg/dl)</th>
<th>Blood HbA1c (%)</th>
<th>Blood GSH (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td></td>
<td>69.3±0.7</td>
<td>5.05±0.2</td>
<td>3.36±0.07</td>
</tr>
<tr>
<td>II (n-saline+ ISO treated i.e. pathogenic control)</td>
<td></td>
<td>93.5±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.90±1.0</td>
<td>1.02±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III (CT-100mg/kg+ ISO)</td>
<td></td>
<td>73.5±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.71±0.1</td>
<td>2.01±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV (AG-200mg/kg+ ISO)</td>
<td></td>
<td>78.2±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.28±0.2</td>
<td>3.01±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V (Gli-25 mg/kg+ ISO)</td>
<td></td>
<td>76.3±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.10±0.2</td>
<td>1.98±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.01, as compared to group I (ANOVA followed by Dunnett's t's t test)
<sup>b</sup>P<0.01, as compared to group II (ANOVA followed by Dunnett's t test)
Table 6.2: Effect aqueous extract of *Clitoria ternatea* (CT-100 mg/kg) and 30% *Aloe Vera Gel* (AG-200 mg/kg) pretreatment on lipid peroxides (TBARS) levels in heart, liver and pancreatic tissue of rats treated with isoproterenol.

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (nmoles MDA/mg protein)</th>
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<tr>
<td></td>
<td></td>
<td>Heart</td>
<td>Liver</td>
<td>Pancreas</td>
</tr>
<tr>
<td>I (Normal healthy Control)</td>
<td></td>
<td>0.53±0.04</td>
<td>0.47±0.03</td>
<td>0.46±0.04</td>
</tr>
<tr>
<td>II (n- saline + ISO treated i.e. pathogenic control)</td>
<td>3.94±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.17±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.87±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>III (CT-100mg/kg + ISO)</td>
<td>1.60±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.28±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IV (AG-200mg/kg + ISO)</td>
<td>0.94±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>V (Gli-25 mg/kg + ISO)</td>
<td>2.54±0.81</td>
<td>2.03±0.05</td>
<td>1.29±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
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</table>

<sup>a</sup> P<0.01, as compared to group I (ANOVA followed by Dunnett's t test)
<sup>b</sup> P<0.05, as compared to group II (ANOVA followed by Dunnett's t test)
<sup>c</sup> P<0.05, as compared to group II (ANOVA followed by Dunnett's t test)
Table 6.3: Effect aqueous extract of *Clitoria ternatea* (CT-100 mg/kg) and 30% Aloe Vera Gel (AG-200 mg/kg) pretreatment on GSH levels in heart, liver and pancreatic tissue of rats treated with isoproterenol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μg/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
<td>Pancreas</td>
</tr>
<tr>
<td>I (Normal healthy Control)</td>
<td>99.6 ±6.0</td>
<td>68.3 ±2.2</td>
<td>45.2 ± 2.9</td>
</tr>
<tr>
<td>II (ISO treated i.e. pathogenic control)</td>
<td>23.01±2.1^a</td>
<td>23.8 ±1.7^a</td>
<td>21.3 ± 2.1^a</td>
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<tr>
<td>III (CT-100mg/kg + ISO)</td>
<td>43.1 ±1.5^b</td>
<td>40.3 ±1.7^b</td>
<td>38.3 ± 3.1</td>
</tr>
<tr>
<td>IV (AG-200mg/kg + ISO)</td>
<td>72.8±1.2^b</td>
<td>46.3 ±1.9^b</td>
<td>42.2 ± 1.7^b</td>
</tr>
<tr>
<td>V (Gli-25 mg/kg + ISO)</td>
<td>36.9 ± 0.7</td>
<td>36.3 ±0.9</td>
<td>34.3 ± 3.1^c</td>
</tr>
</tbody>
</table>

^a P<0.01, as compared to group I (ANOVA followed by Dunnett’s t test)

^b P<0.01, as compared to group II (ANOVA followed by Dunnett’s t test)

^c P<0.05, as compared to group II (ANOVA followed by Dunnett’s t test)
Table 6.4: Effect aqueous extract of *Clitoria ternatea* (CT-100 mg/kg) and 30% *Aloe Vera Gel* (AG-200 mg/kg) pretreatment on catalase levels in heart, liver and pancreatic tissue of rats treated with isoproterenol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (nmol H$_2$O$_2$ –consumed/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>I (Normal healthy Control)</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>II (ISO treated i.e.</td>
<td>0.21±0.03$^a$</td>
</tr>
<tr>
<td>pathogenic control)</td>
<td></td>
</tr>
<tr>
<td>III (CT-100mg/kg + ISO)</td>
<td>1.01±0.01$^c$</td>
</tr>
<tr>
<td>IV (AG-200mg/kg + ISO)</td>
<td>1.96±0.09$^b$</td>
</tr>
<tr>
<td>V (Gli-25 mg/kg + ISO)</td>
<td>0.84±0.41</td>
</tr>
</tbody>
</table>

$^a$P<0.01, as compared to group I (ANOVA followed by Dunnett's t test)

$^b$P<0.01, as compared to group II (ANOVA followed by Dunnett's t test)

$^c$P<0.05, as compared to group II (ANOVA followed by Dunnett's t test)
Table 6.5: Effect pretreatment of aqueous extract of *Clitoria Ternatea* (CT-100 mg/kg) and 30% *Aloe Vera Gel* (AG-200 mg/kg) on SOD levels in heart, liver and pancreatic tissue of rats treated with isoproterenol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (IU/mg protein)</th>
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<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>I (Normal healthy Control)</td>
<td>1.61±0.03</td>
<td>1.73±0.13</td>
<td>0.88±0.04</td>
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<tr>
<td>II (ISO treated i.e. pathogenic control)</td>
<td>0.13±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>III (CT-100 mg/kg + ISO)</td>
<td>0.70±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62±0.07</td>
<td></td>
</tr>
<tr>
<td>IV (AG-200 mg/kg + ISO)</td>
<td>1.03±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.25±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75±0.06</td>
<td></td>
</tr>
<tr>
<td>V (Gl±25 mg/kg + ISO)</td>
<td>0.62±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.59±0.09</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.01, as compared to group I (ANOVA followed by Dunnett's t test)<br>
<sup>b</sup>P<0.01, as compared to group II (ANOVA followed by Dunnett's t test)<br>
<sup>d</sup>P<0.05, as compared to group I (ANOVA followed by Dunnett's t test)
Figure 6.3 (A-E): Photomicrograph of control, ISO treated rats heart section and protection by C. ternatea and Aloe vera gel (A) Control (B) Isoproterenol treated (C) AG-200 mg/kg treated rat following with ISO for two subsequent days (D) CT-100 mg/kg treated rat following with ISO for two subsequent days (E) Gliclazide 25 mg/kg treated rat following with ISO for two subsequent days.
Figure 6.4 (A-E): Photomicrograph of control, ISO treated rats liver section and protection by C. ternatea and Aloe vera gel (A) Control (B) Isoproterenol treated (C) AG- 200 mg/kg treated rat following with ISO for two subsequent days (D) CT-100 mg/kg treated rat following with ISO for two subsequent days (E) Gliclazide 25 mg/kg treated rat following with ISO for two subsequent days.
Figure 6.5 (A-E): Photomicrograph of control, ISO treated rats pancreatic section and protection by C. ternatea and Aloe vera gel (A) Control (B) Isoproterenol treated (C) AG-200 mg/kg treated rat following with ISO for two subsequent days (D) CT-100 mg/kg treated rat following with ISO for two subsequent days (E) Gliclazide 25 mg/kg treated rat following with ISO for two subsequent days.
DISCUSSION

Isoproterenol, a β- adrenergic agonist and synthetic catecholamine, depletes the energy of cardiac muscle and cause complex biochemical and structural changes leading to cell damage and necrosis (Karthick et al., 2006). The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial ischemia/ infraction (Senthil et al., 2007). Isoproterenol is poorly absorbed from the stomach but it is well absorbed from small intestine and proximal colon (Conolly et al., 1972; Conway et al., 1968), thus oral route is not very effective and is no longer recommended. It is now well recognized that the toxic doses of catecholamine produce myocardial necrosis. Various mechanisms are proposed to explain the reason behind this induction of necrosis, that include an increase in cAMP levels (Bhagat et al., 1978; Dhalla et al., 1980), intracellular calcium overload, and exhaustion of high energy phosphates (Fleckenstein et al., 1974). As catecholamine readily undergo oxidation and it is suggested that oxidation products of catecholamines like adrenochrome, rather than catecholamines per se, are responsible for myocardial damage observed after administration of parent compound (Raab 1943; Yates and Dhalla, 1975). Various reports produced strong evidence that catecholamines and their oxidation metabolites can cause cell necrosis and contractile failure in the rat heart (Beamish et al., 1981; Yates et al., 1981; Singal, Yates et al., 1981). Since, isoproterenol has a chronotropic effect on heart, it give rise to heart rate and it also has vasodilating effects, thus reduces the BP. ISO administrations associated with pronounced metabolic abnormalities in blood glucose levels. Increase in the level of blood glucose was observed in ISO-induced rats. An increase in the levels of blood glucose is due to enhance glycogen breakdown and less utilization by the peripheral tissue in MI in rats (Prabu et al., 2006; Rajadurai et al., 2007). Our data represented here clearly show that administration of a synthetic catecholamine isoproterenol caused
a rise in blood glucose level and heart rate and fall in mean blood pressure in rats. Similar finding were reported by various authors (Nirmala C., 1996; Nandave et al., 2007). This increase in blood glucose level, heart rate also give rise to formation of free radical for further induction of damage to normal tissues. In our study administration of Aloe vera gel and aqueous extract of Clitoria ternatea showed a protection via control on blood glucose level and they also maintained the heart rate and blood pressure towards healthy control rats. Preadministration of C. ternatea and 30% Aloe vera gel maintained the blood sugar level showed by blood HbA1c level.

The deleterious role of free radicals (FRs) in myocardial damage induced by ischemia is well established. A growing body of evidence demonstrates increased rate of production of FRs in ischemic heart disease. Under normal physiological condition, formation of FRs is limited by endogenous antioxidant defense system. In ischemia heart disease, the production of FRs is increased at a rate that overwhelms the capacity of endogenous antioxidant defense system fro detoxification, thereby resulting in FR mediated oxidative damage (Cuzzocrea et al., 2001). It is also known that autoxiation of catecholamines results in the generation of highly cytotoxic free radicals (Cohen and Heikkila, 1974; Sachs and Jonsson, 1975; Graham et al., 1978). It is therefore likely that free radicals may play an important role in catecholamine- induced cardiotoxicity by causing peroxidation of membrane phospholipids which can result in permeability changes in the membrane as well as intercellular calcium overload. Rat administered with isoproterenol showed a significant rise in serum LDH and CK level. Various authors have also reported (Shashikumar and Devi., 2000; Manjula et al., 1992) the significant decrease in heart LDH and CK level with significant increase in serum LDH and CK level after administration of isoproterenol when compared to isoproterenol and treatment with abana and aspirin minimized the changes. An increase in the
activity of marker enzymes in serum could be due to the leakage of enzyme from heart as a result of isoproterenol induced necrosis (Paritha et al., 1996) and the amount of enzyme in serum is in proportion to number of necrotic cells (Geetha et al., 1990). In our study administration of Aloe vera gel and Clitoria ternatea significantly reduce the level of LDH and CK in serum when compared to isoproterenol treated rats and this way provides a protection. Myocardial infraction is accompanied by the disintegration of membrane polyunsaturated fatty acid expressed by increase of thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxides and by the impairment of natural scavenging enzymes, characterized by the decrease in the level of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, reduced glutathione (GSH) (Nirmala and Puvanakrishnan., 1996). Rat administered with ISO showed a significant increase in TBARS levels of heart, liver and pancreas that could be due to involvement of free radicals in membrane damage. Administration of Aloe vera gel and C. ternatea maintained the lipid peroxidation level by reducing free radical generation or by protecting antioxidant enzymes. Both the drug may provide protection due to their antioxidant and anti-peroxidative properties.

Oxidative stress can damage many biological molecules and, indeed, proteins and DNA are more often significant targets of injury than are lipids, with lipid peroxidation often occurring late in the injury process (Hallwell and Chirico, 1993). Oxidative stress leads to the formation of free radicals and it is tempting to speculate that catecholamine-induced changes in the heart may involve an increase in free-radical activity. Free radicals can initiate the formation of alkyl, alkoxy, and hydroperoxy radicals plus hydroperoxides from polyunsaturated fatty acids (McCay and King, 1980) and enhance lipid peroxidation. Due to increase in lipid peroxidation, glutathione levels were lowered significantly in blood and in tissue of
isoproterenol treated rats. Decreased glutathione level may be due to its increased utilization in protecting SH groups containing protein from the action of free radicals. Glutathione participated directly in the destruction of hydrogen peroxide and also promotes the formation of reduced form of ascorbate which has high antioxidant activity (Mrtensson and Meister, 1991). Aloe vera gel and Clitoria ternatea pre treatment maintained the level of blood and tissues. Free radical scavenging enzymes such as SOD, catalase and GPx are the first line of defense against oxidative injury. The equilibrium between these enzymes is an important factor for the effective removal of ROS in intracellular organelles (Andrew and Mathew, 1989). Isoproterenol treated rats’ showed decreased activity of SOD and catalase in heart, liver and pancreas. A decrease in the activity of these antioxidant enzymes can lead to the formation of oxygen and hydrogen peroxide, which in turn can form the toxic hydroxyl radical (OH). The decrease in activity of SOD and catalase may be due to myocardial cell damage (Karthick and Prince, 2006). The increased activity of tissue SOD and catalase is associated with decreased levels of lipid peroxidation in Clitoria ternatea and Aloe vera gel treated animals. Histopathological studies also showed the protection of vital organs by administration of Clitoria ternatea and Aloe vera gel. All these results showed that the administration of C. ternatea and Aloe vera gel in rats results in decreased formation of toxic intermediates via reduction in free radical generation and maintain the free radical scavenging enzyme levels. All the results are much significant with Aloe vera gel.

All these biochemical findings support the histological findings of our study, in heart tissue pretreatment with both the drugs provide protection in cellularity and myocardium, 30 % Aloe vera gel showed an almost normal myofibril structure with striations while C. ternatea showed normal myofibril structure with some leucocyte infiltrate. In pancreatic these drugs protect
pancreatic cells by damage induced by isoproterenol, similarly in liver section pretreatment with 30 % Aloe vera gel showed normal cytoplasm and mild inflammation, Clitoria ternatea also provide protection it showed shrunken nuclei with mild granulation in cytoplasm. Aloe vera gel provides a better protection compared to aqueous extract of C. ternatea.