Chapter 2: Ellagic acid ameliorates electrocardiographical, blood pressure and biochemical alterations in myocardial infarction.

2.1. Background

Myocardial infarction is the common presentation of CVD. It occurs when myocardial ischaemia surpasses the critical threshold level for an extended time resulting in irreversible myocardial cell damage (Patel et al., 2010). Electrophysiological, hemodynamic changes and elevated serum levels of markers such as cardiac troponins, myoglobins, creatine kinase (CK) and creatine kinase-MB (CK-MB) are detectable with myocardial infarction. Elevated levels of inflammation related markers, including C-reactive protein (White et al., 2008) are also considered as standard biomarkers of CVD. Various experimental and clinical studies have shown that enormous amounts of ROS such as, superoxide, hydrogen peroxide and hydrogen radicals are generated in failing myocardium (Rajadurai and Stanely Mainzen Prince, 2006). As a defence mechanism against the toxic ROS, cells including cardiomyocytes are provided with non-enzymatic antioxidants such as glutathione, vitamin E, and ascorbic acid and enzymatic antioxidants such as SOD, catalase, and GSH-Px (Carletti et al., 2007). These enzymes are in the prevention and repair of free-radical generated tissue damage, in a pathological condition. The recognition that free radicals mediate myocardial injury has created opportunities to interrupt the injury cascade and preserve the myocardium at risk using antioxidants (Singal et al., 1998). In this chapter, we discussed the role of ellagic acid, a polyphenolic antioxidant against pathological changes in electrocardiogram, heart rate, blood pressure, biochemical parameters such as cardiac markers, lipid peroxidation products and antioxidant enzymes in the isoproterenol-induced myocardial infarction using male Wistar rats.
2.2. Materials and methods

2.2.1. Chemicals and Reagents

Ellagic acid, isoproterenol hydrochloride, p-nitrophenyl-N-acetyl-b-D-glucosaminide, and p-nitrophenyl-b-D-glucuronide were purchased from Sigma Chemical Co., St. Louis, MO, USA. Xylenol orange, thiobarbituric acid, 1, 1, 3, 3’-tetramethoxy propane and sodium pyrophosphate, N-phenyl-p-phenylene diamine, dithionitrobenzoic acid, nitrobluetetrazolium, phenazinemethosulphate, disodium hydrogen phosphate, oxidized glutathione, 1-chloro-2, 4-dinitrobenzene and oxidized glutathione were obtained from S.D. Fine Chemicals, Mumbai, India. Tyrosine was purchased from Himedia Laboratories Private Limited, Mumbai, India. All other chemicals used in this study were of analytical grade.

2.2.2. Experimental animals

All the experiments were done on male albino Wistar rats weighing 180-200 g, purchased from Mahaveer Enterprises, Hyderabad, India. They were housed in polypropylene cages (47 × 34 × 20 cm) lined with husk, renewed every 24 h under a 12 h light/dark cycle at around 22 °C with 50% humidity. The rats had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Pune, Maharashtra, India) once a day. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Institutional Animal Ethical Committee of Jayamukhi College of Pharmacy (Approval No: 20/IAE/J; Dated 05-04-2010).

2.2.3. Induction of experimental myocardial infarction

Isoproterenol hydrochloride (100 mg/kg) was dissolved in saline and subcutaneously injected to rats at intervals of 24 h for 2 days (Punithavathi and
Stanely Mainzen Prince, 2010). Rats were sacrificed 48 h after the first dose of isoproterenol.

2.2.4. Experimental design

The rats were randomly divided into six groups of six rats each. Group I: normal control rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group II: normal rats were treated with ellagic acid (7.5 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group III: normal rats were treated with ellagic acid (15 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 10 days, Group IV: rats were subcutaneously injected with isoproterenol (100 mg/kg) in 2 ml of saline twice at an interval of 24 h (on the 11th and 12th day); Group V: rats were pretreated with ellagic acid (7.5 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then subcutaneously injected with isoproterenol (100 mg/kg) twice at an interval of 24 h (on the 11th and 12th day); Group VI: rats were pretreated with ellagic acid (15 mg/kg) in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then subcutaneously injected with isoproterenol (100 mg/kg) twice at an interval of 24 h (on the 11th and 12th day).

Acute toxicity studies

The study was performed according to the “Guidelines for Repeated Dose 28-Day Oral Toxicity Study in Rodents (1995; No. 407)” provided by the Organization for Economic Co-operation and Development (OECD) and was conducted in compliance with FDA Good Laboratory Practice Regulations (US Food and Drug Administration, 1978, Part 58 of 21 Code of Federal Regulations). The dose level was selected according to the preliminary toxicity studies in rat.
The rats were divided into six groups of three animals in each group. Ellagic acid was dissolved in an appropriate volume of normal saline and administered orally at doses 250, 500, 1000 mg/kg body weight/day for a period of 28 days. Same volume of normal saline without ellagic acid was given to normal control group. Morphological, behavioral, and toxic symptoms of the animals were observed for 24, 48, and 72 h and the animals were weighed biweekly for the whole treatment period for delayed toxicity.

2.2.5. Electrocardiogram

The rats were trained for a week before recording electrocardiogram. Twenty four hours after the second dose of isoproterenol, the rats of all the groups were anesthetized (Ketamine hydrochloride 100 mg/kg body weight, i.p.), and the electrocardiograph patterns were recorded by a 16 channel polygraph (Biopac systems Inc., USA) with minimal stress. The electrocardiogram is analyzed using a digital acquisition and analysis system (AD Instrument Power Lab). Rectal temperature is continuously monitored and maintained within 37-38 °C using a heat pad and heat lamp. Electrocardiographic transducers were inserted subcutaneously into the right forelimb and each hind limb as mentioned in the AD instruments’ protocol. The signal is acquired for about 3 min using Lab Chart 7.0 for Windows software on Lenovo ThinkPad. The recorded signal was free of noise and electrical interference. Each test takes ~ 10-12 min including anesthetic induction and recovery time. The alterations of ECG (P wave, QRS complex, ST-segment elevation, RR interval) in the normal and experimental rats were recorded. The heart rate was determined from electrocardiograph.
2.2.6. Measurement of blood pressure by non-invasive method

For arterial blood pressure measurements by tail cuff method, rats were trained for one week until the blood pressure was recorded with minimal stress and restraint. The tail was introduced into cuff and the pressure was raised and then slowly released. The cuff pressure when the pulse signal reappears, is intended as the systolic pressure. The cuff pressure when the pulse signals level recovers its initial level, is intended as diastolic pressure.

2.2.7. Preparation of samples for biochemical estimation

After recording the electrocardiogram and measuring blood pressure, the rats were sacrificed by cervical decapitation and blood was collected in two tubes, i.e., one with anticoagulant (ethylene diamine tetra acetic acid) for plasma separation, and another without anticoagulant for serum separation. Both the plasma and serum were separated from each sample and used for the biochemical analysis. Immediately after sacrifice, heart tissues were excised in ice cold condition. They were blotted free of blood and tissue fluids. Then they were weighed and stored at −80 °C till further use for analysis. A known weight of the heart tissue was homogenized in ice chilled 0.1 M Tris-HCl buffer (pH 7.4) in Potter-Elvejhem Teflon homogenizer. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

2.2.8. Estimation of cardiac marker enzymes

Assay of cardiac troponin-T

The level of serum cardiac troponin-T was estimated by chemiluminescence immunoassay, using the standard kit purchased from Roche Diagnostics, Switzerland.
Assay of cardiac troponin-I

Cardiac troponin-I in the serum was estimated, using VITROS immunodiagnostic kits purchased from the Ortho-Clinical Diagnostics, Inc. New York, USA.

Assay of myoglobin

Myoglobin in the serum was estimated, using VITROS immunodiagnostic kits purchased from the Ortho-Clinical Diagnostics, Inc. New York, USA.

Assay of CK

Assay of CK in the serum was estimated by the standard diagnostic kit from Accurex Pvt. Ltd, Mumbai, India.

Assay of CK-MB

Assay of creatine kinase-MB in the serum was estimated by the standard diagnostic kit from Accurex Pvt. Ltd, Mumbai, India.

Assay of lactate dehydrogenase (LDH)

Assay of LDH in the serum was estimated by the standard diagnostic kit from Accurex Pvt. Ltd, Mumbai, India.

Estimation of C-reactive protein.

The C-reactive protein was estimated by immunoassay kit purchased from Chemicon, MA, USA.

Estimation of plasma homocysteine.

The plasma homocysteine concentration was assayed by immunoassay kit obtained from Life technologies (India) Pvt. Ltd., Delhi, India.

Assay of CK in the heart tissue

CK activity in the heart tissue was assayed by the method of Okinaka et al., (1961). The incubation mixture contained 0.75 ml of double distilled water, 0.05 ml
of tissue homogenate, 0.1 ml of ATP solution, 0.1 ml of magnesium cysteine reagent and 0.1 ml of creatine. This mixture was incubated at 37 °C for 20 min. The tubes were centrifuged and the supernatant was used for the estimation of phosphorous by the method of Fiske and Subbarow, (1925). One ml of the supernatant was taken and made up to 4.3 ml with double distilled water and 1ml of ammonium molybdate was added, and incubated at room temperature for 10 min. To this, 0.4 ml of 1-amino-2-naphthol-4-sulfonic acid (ANSA reagent) was added. The colour developed was measured at 640 nm in a UV-Visible spectrophotometer after 20 min. The enzyme activity was expressed as IU/L for serum and μmol of phosphorous liberated /min/mg protein for heart tissue.

**Assay of LDH in the heart tissue**

The activity of LDH in the heart tissue was assayed by the method of King, (1965a). To a set of tubes, 1 ml of the buffered substrate and 0.1 ml of serum/tissue homogenate was added, and incubated at 37 °C for 15 min. The incubation was continued for another 15 min, after adding 0.3 ml of nicotinamide adenine dinucleotide (NAD) solution. The reaction was then arrested by adding 1ml of 2,4-dinitrophenylhydrazine (DNPH) reagent and the tubes were incubated for a further period of 15 min at 37 °C. 0.1 ml of the serum/tissue homogenate was added to the control tubes after arresting the reaction with DNPH. To this 7 ml of 0.4 N sodium hydroxide was added, and the colour developed was measured at 420 nm in a UV-Visible spectrophotometer. The enzyme activity was expressed as IU/L for serum and nmol of pyruvate liberated/min/mg protein for heart tissue.

**2.2.9. Estimation of plasma thiobarbituric acid reactive substances (TBARS)**

The concentration of plasma TBARS were measured by the method of Yagi, (1987). To 0.5 ml of plasma, 4 ml of 0.083 N sulphuric acid was added. To this
mixture, 0.5 ml of 10% phosphotungstic acid was added, and mixed thoroughly. The mixture was centrifuged at 3,000×g for 10 min, after allowing the tubes to stand at room temperature for 5 min. The supernatant was discarded and the sediment was mixed with 2 ml of sulphuric acid and 0.3 ml of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at 3,000×g for 10 min. The sediment was suspended in 4 ml of double distilled water and 1 ml of TBA reagent was added. The reaction mixture was heated at 95 °C for 60 min. 5 ml of n-butanol was added after cooling, and the mixture was shaken vigorously and centrifuged at 3,000×g for 15 min. The colour extracted in n-butanol layer was measured at 530 nm in a UV-Visible spectrophotometer. The values were expressed as nmol/ml plasma.

2.2.10. Estimation of TBARS in heart

The concentration of TBARS in heart tissue was estimated by the method of Fraga et al., (1988). 1 ml of the tissue homogenate was treated with 2 ml of thiobarbituric acid (TBA)- trichloroacetic acid (TCA)- hydrochloric acid (HCl) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 min. The tubes were centrifuged at 3,000×g for 10 min after cooling, and the supernatant was taken for measurement. A series of standard solutions in the concentration range of 2-10 nmol was treated in a similar manner. The absorbance of chromophore was measured at 535 nm against reagent blank in a UV-Visible spectrophotometer. The values were expressed as mmol/100 gm wet tissue.

2.2.11. Estimation of lipid hydroperoxides

Lipid hydroperoxides were estimated by the method of Jiang et al., (1992). 1.8 ml of the Fox reagent was mixed with 0.2 ml of plasma/tissue homogenate and incubated for 30 min at room temperature, and the absorbance was measured at
560 nm in a UV-Visible spectrophotometer. Lipid hydroperoxides were expressed as values×10^{-5} mmol/dl of plasma or mmol/100 gm of wet tissue.

2.2.12. Assay of antioxidant enzymes

**Assay of superoxide dismutase**

SOD activity was assayed by the method of Kakkar *et al.*, (1984). 0.5 ml of tissue homogenate was diluted to 1 ml with double distilled water. Then, 2.5 ml of ethanol and 1.5 ml of chloroform, both chilled, were added. This mixture was shaken at 4 °C and then centrifuged.

To an appropriately diluted 0.2 ml of supernatant, 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of phenazinemethosulphate, 0.3 ml of nitrbluetetrazolium, 0.2 ml of nicotinamide adenine dinucleotide- reduced (NADH) and double distilled water in a total volume of 3 ml. The reaction was started by the addition of NADH. After incubation at 30 °C for 90 s, the reaction was stopped by the addition of 1ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against a butanol blank in a UV- spectrophotometer. A system devoid of enzyme served as control. One unit is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50\% in one minute. The activity was expressed as Units/mg protein.

**Assay of catalase**

The activity of catalase was assayed by the method of Sinha, (1972). To 0.9 ml of phosphate buffer, 0.1 ml of tissue homogenate and 0.4 ml of hydrogen peroxide were added. After 60 s, 2 ml of dichromate acetic acid mixture was added. The tubes were kept in boiling water bath for 10 min and the colour developed was read at 620 nm in a UV-Visible spectrophotometer. Standards in the range of 2-10 μmoles
were taken and preceded as test with blank containing the reagent only. The enzyme activity was expressed as μmol of H$_2$O$_2$ consumed/min/mg protein.

**Estimation of reduced glutathione (GSH)**

Reduced GSH was estimated by the method of Ellman, (1959). A known weight of heart tissue was homogenized in phosphate buffer. From this, 0.5 ml was pipetted out and precipitated with 2 ml of 5% TCA. To 1 ml of the supernatant/0.5 ml of plasma, 0.5 ml of Ellman’s reagent and 3 ml of phosphate buffer were added. The yellow colour developed was measured at 412 nm in a UV-Visible spectrophotometer. A series of standards was treated in a similar manner along with a blank containing 3.5 ml of phosphate buffer. The amount of GSH was expressed as mg/dl plasma or mmol/gm wet tissue.

**Assay of GSH-Px**

GSH-Px was assayed by the method of Rotruck et al., (1973). To 0.2 ml of tris-buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To this mixture, 0.2 ml of GSH followed by 0.1ml of hydrogen peroxide was added. The contents were mixed well and incubated at 37 °C for 10 min along with a tube containing all the reagents except the sample. After 10 min, the reaction was arrested by the addition of 0.5 ml 10% TCA, centrifuged and the supernatant was estimated for GSH by the method of Ellman, (1959). The enzyme activity was expressed as μg of GSH consumed/min/mg protein.

**Assay of glutathione reductase (GR)**

The activity of GR was assayed by the method of Horn and Burns, (1978). To 2 ml of phosphate buffer, 0.1ml of tissue homogenate, 0.1 ml of GSSG, 0.1 ml of FAD and 0.5 ml of EDTA were added. The control contained all the reagents except FAD. The tubes were incubated at 37 °C for 15 min, and then 0.1 ml of NADPH
solution was added to all the tubes. The reaction was monitored at 30 nm for 5 min, and the change in absorbance was measured in a UV-Visible spectrophotometer. The enzyme activity was expressed as µmol of NADPH oxidized/h/mg protein.

**Assay of glutathione S-transferase (GST)**

The activity of GST was assayed by the method of Habig et al., (1981). The reaction mixture containing 1 ml of phosphate buffer, 0.1 ml of CDNB and 0.1 ml of the sample was made up to 3 ml with double distilled water. The reaction mixture was pre-incubated at 37 °C for 15 min. To this, 0.1 ml of reduced GSH was added, and the change in absorbance was measured at 340 nm for 3 min at 30 s intervals in a UV-Visible spectrophotometer. The enzyme activity was expressed as nmol of CDNB-GSH conjugate formed/min/mg protein.

**Estimation of α-tocopherol**

α-tocopherol was estimated by the method of Baker et al., (1980). To 0.1 ml of plasma, 1.5 ml of ethanol and 2 ml of petroleum ether were added, mixed and centrifuged. The supernatant was evaporated to dryness at 80 °C. To this, 0.2 ml of 2, 2’-dipyridyl solution and 0.2 ml of ferric chloride solution were added. They were mixed well and kept in dark for 5 min, and then 2 ml of n-butanol was added. Standard α-tocopherol in the concentration range of 10-100 µg were taken and treated similarly along with a blank containing the reagent only. The intense red colour developed was measured at 520 nm in a UV-Visible spectrophotometer. The values were expressed as mg/dl plasma.

**Estimation of ascorbic acid**

Ascorbic acid was estimated by the method of Omaye et al., (1979). 0.5 ml of plasma/tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 20 min at 3,500 ×g. To 0.5 ml of the supernatant, 0.5 ml of DNPH
reagent was added, and mixed well. The tubes were allowed to stand at room temperature for 3 hours, removed and then placed in ice-cold water. To this, 2.5 ml of 85% sulphuric acid was added, and allowed to stand at room temperature for 30 min. A set of standards containing 10-50 μg of ascorbic acid were taken and processed similarly along with blank containing 0.5 ml of 4% TCA. The colour developed was measured at 530 nm in a UV- spectrophotometer. Ascorbic acid values were expressed as mg/dl plasma or mg/gm wet tissue.

2.2.13. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) using Statistical Package for the Social Science software package version 16.00. Results were expressed as mean ± standard deviation for six rats in each group. p values <0.05 were considered significant. Throughout the report, wherever we have used the word “significant” to describe results, we mean “statistically significant at an alpha level of 0.05 (p<0.05) for the two-sided alternative hypothesis.

2.3. Results

Water consumption of ellagic acid dosed and control groups was similar. There was no treatment-related mortality in animals treated with ellagic acid up to dose of 2000 mg/kg weight, indicating that LD$_{50}$ if any should be higher than this dose. Ellagic acid was found to be safe at all doses.

Electrocardiogram patterns of normal and experimental rats are shown in Figure 2.1 and changes in the duration of each event are mentioned in Table 2.1. Normal control and ellagic acid treated rats showed normal electrocardiogram pattern, whereas isoproterenol-induced rats showed a significant increase in ST segment, QT interval along with a significant decrease in the P wave, QRS complex and RR
interval as compared to the normal control group. Oral pretreatment with ellagic acid in isoproterenol-induced rats (7.5 and 15 mg/kg) showed a significant decrease in ST segment, QT interval along with a significant increase in P wave, QRS complex and RR interval, compared to isoproterenol-induced rats.

Figure 2.2 shows the effect of ellagic acid on the arterial blood pressure of the normal and experimental rats. The isoproterenol-induced rats showed significant decreases in the systolic, diastolic and mean arterial blood pressure as compared to the normal control group. Oral pretreatment with ellagic acid for 10 days, enhanced a decrease of the systolic, diastolic and mean arterial pressure in the isoproterenol-induced rats. The activity of ellagic acid was dose dependant and 15 mg/kg shows a higher activity than 7.5 mg/kg. We observed a significant increase in the heart rate of the isoproterenol-induced rats. The oral pretreatment with ellagic acid reduced the heart rate dose dependently and 15 mg/kg produced a better effect than 7.5 mg/kg.

Table 2.2 shows the effect of ellagic acid on cardiac markers in the normal and isoproterenol-induced myocardial infarcted rats. In the isoproterenol-induced rats, the level of cardiac troponin-T, cardiac troponin-I, myoglobin, C-reactive protein and plasma homocystein were significantly increased in the serum. Oral pretreatment with ellagic acid (7.5 mg/kg and 15 mg/kg) significantly decreased all this markers in the serum of the isoproterenol-induced rats.

CK, CK-MB and LDH are among the classical biochemical markers of myocardial damage and are closely correlated with mortality. We studied the activities of these enzymes in the serum and cardiac tissue homogenate of normal and experimental rats. The results are mentioned in Table 2.3. The isoproterenol-induced rats showed a significant increase in the activities of CK, CK-MB, LDH in the serum, compared to the normal control rats. The oral pretreatment with ellagic acid at 7.5 and
15 mg/kg for a period of 10 days significantly inhibited the release of CK, CK-MB, LDH, in the serum of isoproterenol-induced rats. In the isoproterenol-induced rats, the activities of CK, LDH significantly declined in the heart tissue. Oral pretreatment with ellagic acid (7.5 mg/kg and 15 mg/kg) significantly increased the activity of CK and LDH in the heart tissue of the isoproterenol-induced rats.

Table 2.4 shows the effect of pretreatment with ellagic acid on lipid peroxidation in the normal and experimental rats. The isoproterenol-induced myocardial infarcted rats showed a significant increase in TBARS and lipid hydroperoxides levels in the plasma and heart tissue homogenate, compared to normal control rats. Pretreatment with ellagic acid (7.5 and 15 mg/kg) significantly reduced TBARS and lipid hydroperoxides, compared to rats induced with isoproterenol that reflecting the anti-lipid peroxidative effect of ellagic acid.

The activities of enzymatic antioxidants (SOD and catalase) in the normal and experimental rats are studied. The isoproterenol-induced myocardial infarcted rats exhibited a significant decrease in the activities of enzymatic antioxidants level in the heart as compared to the normal control rats. The oral pretreatment with ellagic acid counteracted the deleterious effect of the isoproterenol dose dependently, by increasing the activity of SOD and catalase significantly compared to the rats, which received isoproterenol alone (Table 2.5).

Table 2.6 shows the amounts/activities of reduced GSH in the plasma and heart tissue homogenates, and GSH dependant enzymatic antioxidants such as GSH-Px, GR, GST in the heart tissue homogenates of the normal and isoproterenol-induced rats. The isoproterenol-induced rats exhibited a significant decrease in the amount of reduced glutathione, and activities of reduced GSH dependant enzymatic antioxidants, compared to the normal control rats. The oral pretreatment with ellagic acid (7.5 and
15 mg/kg) to the isoproterenol-induced rats significantly increased the activities of these enzymes, compared with the isoproterenol-alone-induced rats.

Rats induced with isoproterenol exhibited a significant decrease in the amount of α-tocopherol and ascorbic acid in the plasma, compared to the normal control rats. The oral pretreatment with ellagic acid (7.5 and 15 mg/kg) to the isoproterenol-induced rats significantly increased the amount of α-tocopherol and ascorbic acid in the plasma, compared to the isoproterenol-alone-induced rats (Table 2.7).

For all the biochemical parameters studied, ellagic acid at a dose of 15 mg/kg showed the highest significant effect. Rats treated with ellagic acid (7.5 mg/kg and 15 mg/kg) daily for a period of 10 days did not show any toxic effect, hence it is not toxic.
Figure 2.1. Effect of ellagic acid on electrocardiographic pattern in normal and experimental rats.

(a)-Group I: Normal control rats; (b)-Group II: Normal rats + ellagic acid (7.5 mg/kg); (c)-Group III: Normal rats + ellagic acid (15 mg/kg); (d)-Group IV: Isoproterenol-induced rats (100 mg/kg); (e)-Group V: Ellagic acid (7.5 mg/kg) + isoproterenol; (f)-Group VI: Ellagic acid (15 mg/kg) + isoproterenol.
Table 2.1. Effect of ellagic acid on electrocardiographic parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>P wave (s)</th>
<th>QRS interval (s)</th>
<th>ST elevation (mV)</th>
<th>RR interval (s)</th>
<th>Heart Rate (BPM)</th>
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<tr>
<td>Normal control</td>
<td>0.0362±0.00029&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0258±0.00585&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2112±0.0062&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1392±0.0052&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>374±14.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>0.0364±0.00400&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0225±0.00274&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1760±0.0038&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1401±0.0055&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>369±12.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15 mg/kg)</td>
<td>0.0346±0.0055&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>0.0242±0.00376&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1701±0.0034&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1419±0.0055&lt;sup&gt;a&lt;/sup&gt;</td>
<td>363±11.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoproterenol-alone</td>
<td>0.0195±0.00275&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0154±0.0024&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5280±0.0073&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1170±0.0048&lt;sup&gt;b&lt;/sup&gt;</td>
<td>482±22.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg) + isoproterenol</td>
<td>0.0293±0.00291&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0204±0.00246&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3989±0.0057&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1286±0.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>426±23.69&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg) + isoproterenol</td>
<td>0.0337±0.00275&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>0.0238±0.0031&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>0.2229±0.0053&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1348±0.0064&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>381±10.56&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Values are expressed as mean ± standard deviation for six animals in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p<0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days. The ECG parameters are expressed in seconds (s), heart rate as beats per minutes (BPM), ST elevation in milli volt (mV).
Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other ($p < 0.05$, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
Table 2.2. Effect of ellagic acid on cardiac markers in normal and isoproterenol-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Troponin T (ng/ml)</th>
<th>Troponin I (ng/ml)</th>
<th>Myoglobin (ng/ml)</th>
<th>C-reactive protein (mg/ml)</th>
<th>Plasma homocystein (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.49 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.72 ± 2.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.90± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>0.53 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.90± 2.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.12± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.61± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15mg/kg)</td>
<td>0.53 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.33 ± 2.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.12± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.55± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoproterenol-alone</td>
<td>1.72 ±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04 ±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.01 ± 4.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.17± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.33± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg)+isoproterenol</td>
<td>0.96 ±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75 ±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.85 ± 3.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.83± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.60± 0.90&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg)+isoproterenol</td>
<td>0.66 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.48 ±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35.63 ± 2.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.82 ± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.65± 0.46&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p <0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
Table 2.3. Effect of ellagic acid on the activities of creatine kinase (CK), creatine kinase-MB (CK-MB), Lactate dehydrogenase (LDH), in the serum and heart tissue homogenate of normal and isoproterenol-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum CK IU/l</th>
<th>Serum CK-MB IU/l</th>
<th>Serum LDH IU/l</th>
<th>Heart CK (µmol of phosphorus liberated/min/mg protein)</th>
<th>Heart LDH (nmol of pyruvate liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>18.64±1.74(^a)</td>
<td>10.35±1.08(^a)</td>
<td>84.29±7.61(^a)</td>
<td>14.17±1.03(^a)</td>
<td>113.99±11.70(^a)</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>19.24±1.72(^a)</td>
<td>10.13±1.12(^a)</td>
<td>79.56±7.16(^a)</td>
<td>14.72±1.35(^a)</td>
<td>115.07±11.62(^a)</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15mg/kg)</td>
<td>19.16±1.86(^a)</td>
<td>10.02±1.01(^a)</td>
<td>77.51±6.74(^a)</td>
<td>14.17±1.45(^a)</td>
<td>114.62±11.13(^a)</td>
</tr>
<tr>
<td>Isoproterenol-alone</td>
<td>36.75±3.41(^b)</td>
<td>26.56±1.89(^b)</td>
<td>163.39±17.56(^b)</td>
<td>6.67±0.61(^b)</td>
<td>81.23±7.37(^b)</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg)+ isoproterenol</td>
<td>26.80±1.83(^c)</td>
<td>16.54±1.55(^c)</td>
<td>137.53±9.11(^c)</td>
<td>9.77±0.85(^c)</td>
<td>91.43±8.04(^c)</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg) + isoproterenol</td>
<td>23.52±1.85(^d)</td>
<td>12.38±1.78(^d)</td>
<td>101.60±8.04(^d)</td>
<td>12.24±1.30(^a)</td>
<td>106.07±11.71(^d)</td>
</tr>
</tbody>
</table>

Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p<0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
Table 2.4. Effect of ellagic acid on thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides in normal and isoproterenol-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS</th>
<th>Lipid hydroperoxides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (nmol/ml)</td>
<td>Heart (mmol/100gm wet tissue)</td>
</tr>
<tr>
<td>Normal control</td>
<td>2.31± 0.17\textsuperscript{a}</td>
<td>0.67± 0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>2.21± 0.20\textsuperscript{a}</td>
<td>0.68± 0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15 mg/kg)</td>
<td>2.17± 0.16\textsuperscript{a}</td>
<td>0.68± 0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>Isoproterenol-alone</td>
<td>7.00± 0.42\textsuperscript{b}</td>
<td>1.01± 0.01\textsuperscript{b}</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg)+ isoproterenol</td>
<td>3.38± 0.31\textsuperscript{c}</td>
<td>0.84± 0.06\textsuperscript{c}</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg) + isoproterenol</td>
<td>2.58± 0.25\textsuperscript{d}</td>
<td>0.72± 0.04\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p<0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
**Table 2.5.** Effect of Ellagic acid on the activities of superoxide dismutase (SOD) and catalase in heart of normal and isoproterenol-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units/mg protein)</th>
<th>Catalase (µmol of H₂O₂ consumed/min/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9.34± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.14± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>9.76± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.22± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15mg/kg)</td>
<td>9.86± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.37± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoproterenol -alone</td>
<td>4.59± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.78± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg) + isoproterenol</td>
<td>5.81± 0.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.84± 0.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg) + isoproterenol</td>
<td>8.21± 0.59&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.54± 0.52&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SOD units: One unit is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in one minute. Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p < 0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
Table 2.6. Effect of ellagic acid on glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione s-transferase (GST) in normal and isoproterenol-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH Plasma (mg/dl)</th>
<th>GSH Heart (mmol/gm wet tissue)</th>
<th>GPx Heart (µg of GSH consumed/min/mg protein)</th>
<th>GR Heart (Units/mg protein)</th>
<th>GST Heart (nmol of CDNB*-GSH conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>24.96± 2.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.49± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.78± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.69± 2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>25.03± 2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.67± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.73± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.32± 3.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15mg/kg)</td>
<td>25.21± 2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.67± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.75± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.33± 3.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoproterenol-alone</td>
<td>15.96± 1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.14± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.52± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.71± 1.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg) + isoproterenol</td>
<td>18.60± 1.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.45± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.97± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.53± 1.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.56± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg) + isoproterenol</td>
<td>22.44± 2.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.17± 0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.60± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.28± 2.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.91± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

GR units: µmol of NADPH oxidized/hr/mg protein. CNDB*: 1-chloro-2,4-dinitrobenzene. Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p<0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
Table 2.7. Effect of ellagic acid on α-tocopherol and vitamin-C in normal and isoproterenol-induced myocardial infarcted rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>α-Tocopherol Plasma (mg/dl)</th>
<th>Vitamin-C Plasma (mg/dl)</th>
<th>Vitamin-C Heart (mg/gm wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.25± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.31± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>259.10± 20.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (7.5 mg/kg)</td>
<td>2.22± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.38± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.50± 18.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal+ ellagic acid (15mg/kg)</td>
<td>2.18± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.43± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.37± 18.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoproterenol-alone</td>
<td>0.63± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>147.83± 13.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (7.5 mg/kg) + isoproterenol</td>
<td>1.09± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.64± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>187.87± 18.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid (15 mg/kg) + isoproterenol</td>
<td>1.85± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.20± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>245.40± 23.10&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is mean ± standard deviation for six samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly with each other (p<0.05, Duncan’s multiple range test). Ellagic acid was given to rats daily for a period of 10 days.
2.4. Discussion

Throughout the toxicological experimental period, there were no deaths or treatment-related changes in clinical signs were observed. Since there were no differences among the groups, the intake of ellagic acid at various doses was not harmful to the rats.

Increased generation of cytotoxic free radicals by auto-oxidation of isoproterenol subsequently damaging the cardio myocytes and affected the normal cardiac activities. In the electrocardiogram of rats, we observed a decrease in the QRS complex, an elevated ST-segment and a hyper acute T wave with an asymmetric configuration in isoproterenol-induced rats. This may be due to the reduced capacity of ventricular contraction. The ST-segment elevation is the most sensitive marker for myocardial infarction, and it reflects myocardial necrosis and the consequent loss of cell membrane in an injured myocardium (Holland and Brooks, 1977; Kela et al., 1980). The T wave represents the repolarization time, and a prolonged T wave may be due to a delay in recovery and the depleted energy level in the ischemic tissue. The oral pretreatment with ellagic acid significantly reduced the pathological alterations including ST-segment elevation and pathological Q wave. Normalization of the ST-segment by ellagic acid indicates adequate perfusion throughout the myocardial microvasculature and not just the major coronary vessels. These results showed the cardioprotective effect of ellagic acid against isoproterenol-induced myocardial infarction.

Chronic stimulation of isoproterenol down regulated the β₁ and β₂ receptors and reduced arterial pressures in isoproterenol-induced rats. The supra-maximal dose of isoproterenol also produced increased heart rate (tachycardia) due to the involvement of baroreceptor reflex-mediated changes in autonomic nerve activity. Isoproterenol
produces positive inotropic effect on the heart and increased the heart rate. As β-adrenergic receptors are one of the most susceptible receptors to phenolic binding (Zhu et al., 1997), ellagic acid which has been given orally can bind with them and significantly improved the arterial blood pressures and controlled the increased heart rate in the isoproterenol-induced rats.

Cardiac troponins are regulatory proteins that control the calcium-mediated interaction of actin and myosin, which results in contraction and relaxation of striated muscle. An elevated troponin level predicts the risk of both cardiac death and subsequent infarction. Myoglobin represents an intracellular fatty acid transporter (Sriram et al., 2008). The presence of myoglobin in the serum helps to predict myocardial infarct size, either alone or in combination with other cardiac markers. The release of troponins and myoglobin after myocardial infarction correlated with the severity of infarction in experimental rat models and it can be used for the early detection of cardiotoxic and/or cardiodegenerative effects in animals (Remppis et al., 2000). In this study, increased cardiac troponin-T, cardiac troponin-I and myoglobin levels were observed in the serum of isoproterenol-induced myocardial infarcted rats. Cytotoxic free radicals produced by isoproterenol may involve damage to myocytes and the release of troponins and myoglobin into the serum. Oral pretreatment with ellagic acid significantly reduced the level of troponins and myoglobins in the serum of isoproterenol-induced myocardial infarcted rats, evidences its capability to control the free radical attack and protect the myocardium from ischaemia and necrosis.

The increased level of C-reactive protein in the serum of isoproterenol-induced myocardial infarcted rats reflects extend of myocardial necrosis. The oral pretreatment
with ellagic acid (7.5 and 15 mg/kg) significantly reduced the elevated levels of C-reactive protein in the isoproterenol-induced rats. A study by Corbett et al. (2010) reported the anti-inflammatory effect of ellagic acid and its interaction with known cyclooxygenase inhibitors. This known potential mechanism of ellagic acid is dependable with the results of this study.

Homocysteine a product of methionine metabolism, a strong and independent risk factor for CVD was significantly increased in the plasma of the isoproterenol-induced rats, compared to normal control rats. This is in accordance with previous reports (Hagar, 2002). Deficiencies in the enzymes or vitamin cofactors required for homocysteine metabolism may be one of the possibilities for the enhanced level of homocysteine in the systemic circulation. Homocysteine is also a potent inducer of inflammatory processes in endothelial cells at the level of gene expression (Shai et al., 2004). Our finding of increased homocysteine level is incorporated with an increased level of inflammatory marker, C-reactive protein. The oral pretreatment with ellagic acid inhibited the level of homocysteine in the isoproterenol-induced rats. This effect revealed the anti-inflammatory property of ellagic acid.

CK and LDH are the diagnostic markers used to determine cardiac damage. The marker enzymes in the heart tissue homogenates decreased significantly in isoproterenol-induced rats, compared to normal control rats. In clinical diagnosis, the detection of myoglobin and CK-MB isoforms in the serum is helpful in achieving an accurate and specific conclusion to myocardial infarction (Bock et al., 1999; Li et al., 2006). Myoglobin represents an intra cellular fatty acid transporter (Sriram et al., 2008). The presence of myoglobin in the serum helps to predict myocardial infarct size, either alone
or in combination with other cardiac markers. There are a few reports in which myoglobin and CK-MB isoforms have been compared for early diagnosis of myocardial infarction (Mair et al., 1995). The elevated levels of cardiac markers in isoproterenol-induced myocardial infarcted rats exposed the changes in membrane integrity and disturbances in the permeability of the cardiomyocytes. This was due to the enzymes leaking out of the damaged tissues when the cell membrane became permeable or was ruptured during isoproterenol-induced necrotic damage of the myocardial membrane (Sabeena Farvin et al., 2004). Oral pretreatment with ellagic acid increased this enzymes level in the heart tissue of the isoproterenol-induced rats, suggesting its ability to maintain membrane integrity, thereby restricting the outflow of these enzymes from the heart tissue into the circulatory system of myocardial infarcted rats. Ellagic acid contains four hydroxyl groups and two lactone groups. The former are known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage (Pari and Sivasankari, 2008). Ellagic acid inhibits the generation of superoxide and hydroxyl free radicals in both enzymatic and nonenzymatic systems by means of its metal-chelating property, thus providing protection against lipid peroxidation (Yüce et al., 2008). Based on its structure, ellagic acid can interact with cell membranes, regulating the permeability of the cells and can activate or deactivate proteins and transcription factors by being involved in key metabolic pathways.

The degree of lipid peroxidation in the normal and experimental rats was assessed by estimating TBARS and lipid hydro peroxides, the products of lipid peroxidation. Kumaran and Stanely Mainzen Prince (2010a), reported the involvement of lipid peroxidation in the pathogenesis of isoproterenol-induced myocardial infarction.
We observed increased TBARS and lipid hydro peroxides levels in the heart tissue of isoproterenol-induced rats. The increased levels of these products confirmed that there is excess lipid peroxidation in the isoproterenol-induced rats. The increase in lipid peroxidation in isoproterenol-induced rats is a consequence of higher levels of free radicals, which are produced by oxidized metabolic products of isoproterenol. An enormous amount of super oxide radicals formed by isoproterenol can stimulate the Haber-Weiss reaction for further generation of ROS, initiating lipid peroxidation (Becker, 2004). Pretreatment with ellagic acid significantly decreased TBARS and lipid hydroperoxides levels in the systemic circulation, which is reflected in the anti-lipid peroxidation effect of pretreatment with ellagic acid. Pretreatment with ellagic acid significantly reduced TBARS and lipid hydro peroxides levels in the heart tissue. This effect showed the anti-lipid peroxidation property of ellagic acid. The result of this study are in line with previous studies which indicated that ellagic acid had a strong antioxidant effect (Festa et al., 2001), and inhibited lipid peroxidation (Osawa et al., 1987).

The decreased activities of antiperoxidative enzymes, SOD and catalase in the isoproterenol-induced rats were observed. These enzymes are utilized for scavenging super oxides and hydrogen peroxides which are produced by excessive dose of isoproterenol. The amount of reduced GSH and the activities of GSH-Px, GST and GR, in the isoproterenol-induced myocardial infarcted rats were significantly decreased, compared to the normal control rats. The reduced GSH levels might be due to its increased consumption by the myocardium, in protecting thiol containing proteins from lipid peroxides. The unavailability of reduced GSH may decrease the activities of
reduced GSH, GSH-Px, and GST and GR in the isoproterenol-induced myocardial
infarcted rats (Rajadurai and Stanely Mainzen Prince, 2006). The inactivation of GR in
the heart leads to the accumulation of oxidized glutathione, the oxidized product of
reduced GSH (Padmanabhan and Stanely Mainzen Prince, 2006). The decreased levels of
GST reduced the metabolism of the xenobiotic isoproterenol and enhanced the
concentration in the circulatory system that lead to toxicity. The oral pretreatment with
ellagic acid increases the activities of SOD, catalase, reduced GSH, GSH-Px, GST, and
GR in the heart tissue homogenate of the isoproterenol-induced rats. These
pharmacological actions of ellagic acid could considerably develop cellular antioxidant
defence against oxidative stress and protect myocardial tissues.

The decrease in α-tocopherol could be due to the increased consumption of
α-tocopherol in scavenging the oxy radicals generated, or due to decreased ascorbic acid
concentration as there is a well-established interaction between α-tocopherol and ascorbic
acid. The antioxidant property of ellagic acid significantly increased the concentration of
α-tocopherol and ascorbic acid in isoproterenol-induced rats.

The results discussed in this chapter provide basic experimental evidence that the
oral pretreatment with ellagic acid (7.5 and 15 mg/kg) was safe and highly protective
against experimentally induced myocardial infarction. These findings are rational to
understand the beneficial effects of ellagic acid on cardio protection against myocardial
infarction, in which oxidative stress was known to contribute to the myocardial damage.