European Journal of Pharmacology 659 (2011) 45–52

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

Ellagic acid ameliorates isoproterenol induced oxidative stress: Evidence from electrocardiological, biochemical and histological study

M. Mari Kannan [a,b], S. Darlin Quine [c,*]

[a] SASTRA University, Thirumalaisamudram, Thanjavur, Tamil Nadu, India
[b] Department of Pharmacology, Jayamukhi College of Pharmacy, Narsampet, Warangal, Andhra Pradesh, India
[c] Post Graduate and Research Department of Chemistry, Government Arts College, C.Mutlur, Chidambaram, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 9 December 2010
Received in revised form 22 January 2011
Accepted 14 February 2011
Available online 5 March 2011

Keywords:
Ellagic acid
Cardiac marker
Lipid peroxidation
Antioxidant
Isoproterenol

ABSTRACT

The present study was designed to evaluate the cardioprotective effects of ellagic acid against isoproterenol induced myocardial infarction in rats by studying electrocardiography, blood pressure, cardiac markers, lipid peroxidation, antioxidant defense system and histological changes. Male Wistar rats were treated orally with ellagic acid (7.5 and 15 mg/kg) daily for a period of 10 days. After 10 days of pretreatment, isoproterenol (100 mg/kg) was injected subcutaneously to rats at an interval of 24 h for 2 days to induce myocardial infarction. Isoproterenol administered rats showed significant changes in the electrocardiogram pattern, arterial pressure, and heart rate. Isoproterenol-induced rats also showed significant ($P<0.05$) increase in the levels of serum troponin-I, creatine kinase, lactate dehydrogenase, C-reactive protein, plasma homocysteine, heart tissue thiobarbituric acid reactive substances and lipid hydro peroxides. The activities/levels of antioxidant system were decreased in isoproterenol-induced rats. The histopathological findings of the myocardial tissue evidenced myocardial damage in isoproterenol induced rats. The oral pretreatment of ellagic acid restored the pathological electrocardiographic patterns, regulated the arterial blood pressures and heart rate in the isoproterenol induced myocardial infarcted rats. The ellagic acid pretreatment significantly reduced the levels of biochemical markers, lipid peroxidation and significantly increased the activities/levels of the antioxidant system in the isoproterenol induced rats. An inhibited myocardial necrosis was evidenced by the histopathological findings in ellagic acid pretreated isoproterenol induced rats. Our study shows that oral pretreatment of ellagic acid prevents isoproterenol induced oxidative stress in myocardial infarction.

1. Introduction

Accounting for more deaths worldwide, cardiovascular disease is both a familial and lifestyle disorder. Myocardial infarction is the common presentation of cardiovascular disease. It occurs when myocardial ischemia surpasses the critical threshold level for an extended time resulting in irreversible myocardial cell damage (Patel et al., 2010). Various experimental and clinical studies have shown that enormous amounts of reactive oxygen species such as, superoxide, hydrogen peroxide and hydrogen radicals are generated in failing myocardium (Rajadurai and Stanely Mainzen Prince, 2006). The recognition that free radicals mediate myocardial injury has created opportunities to interrupt the injury cascade and preserve the myocardium at risk (Singal et al., 1982). As a defense mechanism against the toxic reactive oxygen species, cells are provided with non-enzymatic (glutathione, vitamin E, and ascorbic acid) and enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase (Carletti et al., 2007).

Isoproterenol, a synthetic catecholamine and a β-adrenoceptor agonist, has been found to induce myocardial infarction in rats (Rathore et al., 1998). The generation of highly cytotoxic free radicals through the auto-oxidation of catecholamines and a disturbance in the physiological balance between production of free radicals and an anti-oxidative defense system (Srivastava et al., 2007), have been implicated as the important risk factors in the loss of integrity and function of myocardial membranes. The pathophysiological and morphologic alterations in the heart of this non-coronary myocardial necrotic rat model are comparable with those taking place in human myocardial infarction (Anandan et al., 2007; Panda and Naik, 2008; Zhou et al., 2008).

In recent years, polyphenols have attracted considerable attention as agents that protect cells or molecules from oxidative myocardial injury. Ellagic acid is one such polyphenolic phytonutrient found in wide varieties of berries and nuts, and it has received particular attention because of its extensive array of biological properties. Previous studies indicated that ellagic acid showed free radical scavenging action (Priyadarsini et al., 2002), chemoprotective, anti-inflammatory, anti-fibrotic activities (Vattem and Shetty, 2005; Corbett et al., 2010;

* Corresponding author. Tel./fax: +91 4144 292159.
E-mail address: darlinmainzen728@gmail.com (S.D. Quine).

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doi:10.1016/j.ejphar.2011.02.037

Devipriya et al., 2008) and inhibited lipid peroxidation (Osawa et al., 1987). Current gold standard markers of cardiovascular disorders include electrophysiological changes and elevated serum levels of cardiac specific proteins. Cardiac troponins are detectable with myocardial infarction and inflammation-related proteins, including C-reactive protein, at elevated levels with heart failure (White et al., 2008).

Polyphenols are excellent cadioprotectants. Hence, we hypothesized that ellagic acid may have a cardioprotective effect. The objective of the present study was designed to investigate the protective effect of the oral pretreatment of ellagic acid on experimentally induced myocardial infarction in Wistar rats. This study also attempted to explain the possible mechanism of the ellagic acid, by studying the electro cardiological, hemodynamic, biochemical changes in isoproterenol induced model of myocardial infarction.

2. Materials and methods

2.1. Drug and chemicals

Ellagic acid and isoproterenol hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, USA. Xylenol orange, dithionitro bis benzoic acid, nitroblue tetrazolium, phenazine methosulphate and oxidized glutathione were obtained from S.D. Fine Chemicals, Mumbai, India. All other chemicals used in this study were of analytical grade.

2.2. Experimental animals

All the experiments were carried out with 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. 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.

2.3. Induction of experimental myocardial infarction

Isoproterenol (100 mg/kg) was dissolved in saline and subcutaneously injected to rats twice at an interval of 24 h (Punithavathi and Prince, 2010). Animals were sacrificed 48 h after the first dose of isoproterenol.

2.4. Experimental protocols

The rats were divided into six groups of eight rats each. Two rats from each group were used for the histological study. 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 days); 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 days); 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 days).

2.5. Electrocardiogram

Twenty four hours after the second dose of isoproterenol, the rats of all the groups were anesthetized with ketamine hydrochloride (100 mg/kg body weight) intraperitoneally and the electrocardiograph patterns were recorded by a 16 channel polygraph (Biopac systems Inc., USA). The type of alterations (P wave, QRS complex, ST-segment elevation, RR interval) in the normal and experimental rats was recorded.

2.6. Measurement of blood pressure by non-invasive method

Twenty four hours after the second dose of isoproterenol, blood pressures were measured. For arterial blood pressure measurements using tail cuff method, rats were trained for at least one week until the blood pressure was recorded with minimal stress and restraint. The tail was introduced into the 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 signal level recovers its initial level is intended as diastolic pressure. The heart rate was recorded by the pulse analyser.

2.7. Biochemical analysis

After recording the electrocardiogram and measuring the blood pressure, the animals 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 the analysis.

2.7.1. Analysis of cardiac markers

The level of cardiac troponin-I in the serum was estimated, using VITROS immunodiagnostic kit purchased from the Ortho-Clinical Diagnostics, Inc. New York, USA. Creatine kinase and lactate dehydrogenase in the serum were estimated by the standard diagnostic kit from Accurex Pvt. Ltd, Mumbai, India.

2.7.2. Estimation of serum C-reactive protein and plasma homocysteine

The serum C-reactive protein was estimated by immunoassay kit purchased from Chemicon, MA, USA. The plasma homocysteine level was assayed by immunoassay kit obtained from Life technologies (India) Pvt. Ltd., Delhi, India.

2.7.3. Estimation of thiobarbituric acid reactive substances and lipid hydro peroxides

The concentration of thiobarbituric acid reactive substances was estimated according to Fraga et al. (1988). In this method, malondialdehyde and other thiobarbituric acid reactive substances were measured by their reactivity with thiobarbituric acid in acidic conditions to generate a pink coloured chromophore, which was read at 535 nm. Lipid hydro peroxides in heart tissue were estimated by the method of Jiang et al. (1992). In this method, oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange led to the formation of a chromophore, which was read at 560 nm.

2.7.4. Estimation of antioxidant enzymes and reduced glutathione

The clear supernatant obtained from the heart tissue homogenate was used for the assay of endogenous antioxidant enzymes superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, glutathione reductase and the levels of reduced glutathione. Superoxide dismutase was measured by the method of Kakkar et al. (1984). Superoxide radicals react with nitroblue tetrazolium in the presence

of reduced nicotinamide adenine dinucleotide and produce formazon blue. Superoxide dismutase removes the superoxide radicals and inhibits the formation of formazon blue. The intensity of the colour is inversely proportional to the activity of the enzyme and read at 560 nm. Catalase was determined by the method of Sinha (1972). In this method, dichromate in acetic acid is converted to perchromic acid and then to chromic acetate when heated in the presence of hydrogen peroxide. The chromic acetate formed was measured at 620 nm. Reduced glutathione was measured by the method of Ellman (1959). This method is based on the development of yellow colour, when dithionitro benzoic acid is added to compounds containing sulfhydryl groups. The colour developed was read at 412 nm. Glutathione peroxidase was assayed by the method of Rotruck et al. (1973). A known amount of enzyme preparation was allowed to react with hydrogen peroxide and glutathione for a specified time period. The glutathione content remaining after the reaction was measured by Ellman's reaction. The activity of glutathione reductase was assayed by the method of Horn and Burns (1978) by recording the decrease in absorbance due to depletion of NADPH for a period of 5 min at 340 nm. The activity of glutathione-s-transferase was assayed in the cardiac tissue following the increase in the absorbance at 340 nm using 1-chloro-2, 4-dinitro benzene as substrate by the method of Habig and Jakoby (1981).

2.7.5. Estimation of non enzymatic antioxidants

α-Tocopherol was estimated by the method of Baker et al. (1980). This method involves the reduction of ferric ion to ferrous ion by α-tocopherol and the formation of a red coloured complex with 2, 2′-dipyridyl. The absorbance of the chromophore was measured at 520 nm. Ascorbic acid was estimated by the method of Omaye et al. (1979). In this method, the ascorbic acid is converted into dehydro ascorbic acid in the presence of thiourea, a mild reducing agent and then coupled with 2, 4-dinitrophenyl hydrazine. The coupled 2, 4-dinitrophenyl hydrazine is converted into a red coloured complex when treated with sulphuric acid, which was read at 530 nm.

2.8. Histopathology

After the sacrifice of the normal and experimental rats, the heart was rapidly dissected and washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the heart tissue was processed by embedding it in paraffin. Then, the heart tissue was sectioned and stained with hematoxylin and eosin (H&E, 40×). The sections were examined under the light microscope for histopathological changes and photomicrographs were taken.

2.9. Statistical analysis

Statistical analyses were performed by One-way Analysis of Variance followed by Duncan's multiple range tests using a Statistical Package for the Social Science software package version 16.00. The results were expressed as mean ± standard deviation for six rats in each group. The P values <0.05 were considered significant.

3. Results

Electrocardiogram patterns of normal and experimental animals are shown in Fig. 1 and changes in the duration of each event are mentioned in Fig. 2. Normal control and ellagic acid treated rats showed normal electrocardiogram pattern, whereas isoproterenol-induced rats showed a significant ($P<0.05$) increase in ST-segment, QT interval along with a significant ($P<0.05$) 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 ($P<0.05$) decrease in ST segment, QT interval along with a significant ($P<0.05$) increase in P wave, QRS complex and RR interval, when compared to isoproterenol-induced rats.

Fig. 3 shows the effect of ellagic acid on the extent of the histological changes in the myocardial tissues of the normal and isoproterenol induced rats. Fig. 3A shows the light micrograph of the heart in normal rat showing a normal architecture without any infarction. The rats treated with ellagic acid (7.5 and 15 mg/kg) showed normal cardiac muscle bundles without any damage, as shown in Fig. 3B and C. The light micrograph of the isoproterenol-induced group shows the focal confluent necrosis of cardiac muscle fibers with neutrophil infiltration, as seen in Fig. 3D. The ellagic acid (7.5 mg/kg) pretreated group showed a reduced myocardial necrosis with less edema and much less neutrophil infiltration, as shown in Fig. 3E. The ellagic acid (15 mg/kg) treated rat heart shows mild edema with significant reduction in infarction, showing nearer to normal myocardial architecture, as shown in Fig. 3F.

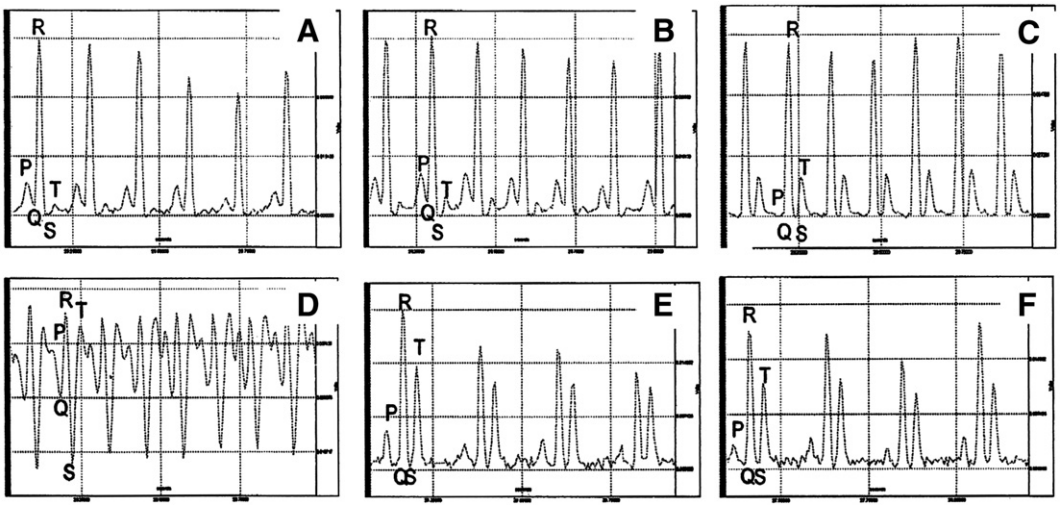

Fig. 1. (A–F): Effect of ellagic acid on electrocardiographic pattern in normal and experimental rats. A. Electrocardiogram pattern of normal control group showing normal cardiograph. B. Electrocardiogram pattern of ellagic acid (7.5 mg/kg) pretreated group rats showing normal cardiograph. C. Electrocardiogram pattern of ellagic acid (15 mg/kg) treated group rats showing normal cardiograph. D. Electrocardiogram pattern of isoproterenol(100 mg/kg)-induced group rats showing pathological changes such as ST-segment elevation. E. Electrocardiogram pattern of ellagic acid (7.5 mg/kg) treated isoproterenol-induced group rats showing minimized ST-segment elevation. F. Electrocardiogram pattern of ellagic acid (15 mg/kg) pretreated isoproterenol-induced group rats showing almost normal cardiograph without any elevation in ST-segment.

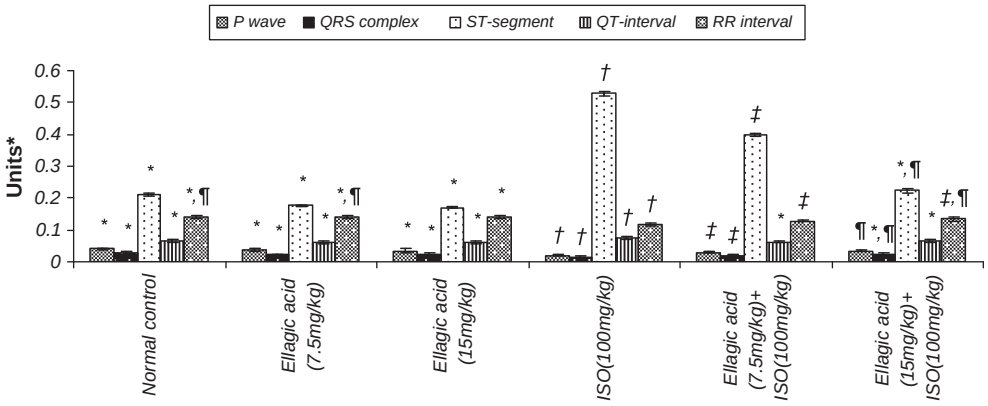

Fig. 2. Changes in the duration of cardiac events. Each column is mean ± standard deviation for six rats in each group; Columns not sharing a common letter (*, †, ‡, ¶) differ significantly with each other ($P<0.05$, Duncan's multiple range test); Normal control group is compared with ellagic acid (7.5 and 15 mg/kg). Isoproterenol-induced (100 mg/kg) group is compared with normal control group; Ellagic acid (7.5 mg/kg) + isoproterenol-induced group and ellagic acid (15 mg/kg) + isoproterenol-induced group are compared with isoproterenol (100 mg/kg). Units*: the electrocardiographic events such as P wave, QRS complex, QT and RR intervals: seconds (s), ST-segment elevation: milli volt (mv).

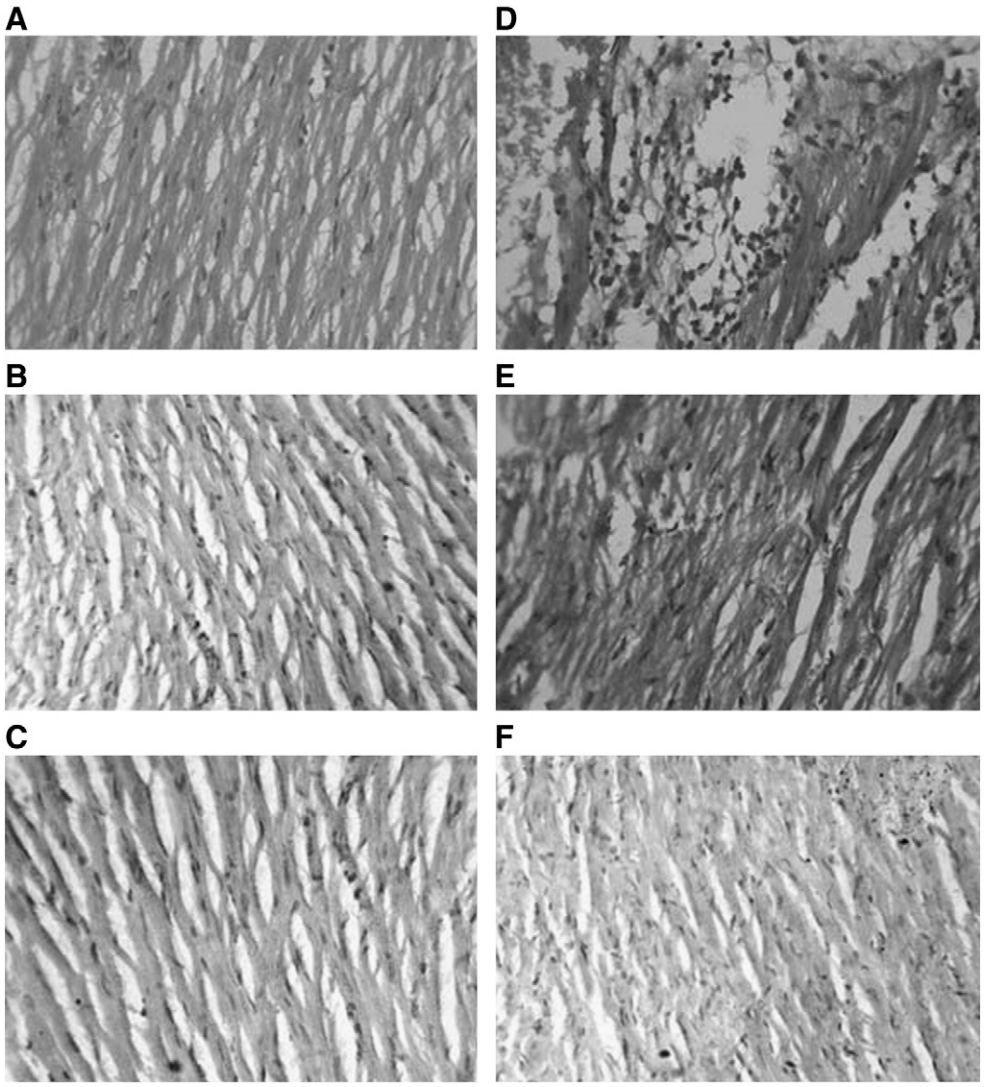

Fig. 3. Histopathology of the heart. A. Microscopic section of normal control group rat heart showing normal architecture of cardiac muscle. B. Microscopic section of ellagic acid (7.5 mg/kg) treated group rat heart showing normal architecture of cardiac muscle. C. Microscopic section of ellagic acid (15 mg/kg) treated group rat heart showing normal architecture of cardiac muscle. D. Microscopic section of isoproterenol-induced group rat heart showing infiltration of inflammatory cells, necrosis and separated cardiac muscle fiber. E. Microscopic section of ellagic acid (7.5 mg/kg) pretreated isoproterenol-induced group rat heart showing decreased infiltration of inflammatory cells and mild separation in cardiac muscle fiber. F. Microscopic section of ellagic acid (15 mg/kg) pretreated isoproterenol-induced group rat heart showing no infiltration of inflammatory cells and normal cardiac muscle fiber.

Table 1
Effect of ellagic acid on arterial blood pressure.

Groups	Systolic pressure (mm Hg)	Mean arterial pressure (mm Hg)	Diastolic pressure (mm Hg)	Heart rate (beats per minute)
Normal control	138.50 ± 7.18^{a}	118.08 ± 7.84^{a}	97.67 ± 8.59^{a}	374 ± 14.53^{a}
Normal + ellagic acid (7.5 mg/kg)	137.33 ± 8.45^{a}	117.75 ± 7.82^{a}	98.17 ± 7.76^{a}	369 ± 12.44^{a}
Normal + ellagic acid (15 mg/kg)	138.00 ± 8.10^{a}	118.50 ± 8.29^{a}	99.00 ± 9.14^{a}	363 ± 11.22^{a}
Isoproterenol-alone (100 mg/kg)	90.67 ± 5.43^{b}	72.75 ± 5.45^{b}	54.83 ± 5.71^{b}	482 ± 22.02^{b}
Ellagic acid (7.5 mg/kg) + isoproterenol (100 mg/kg)	110.67 ± 7.42^{c}	91.58 ± 7.30^{c}	72.50 ± 7.56^{c}	426 ± 23.69^{c}
Ellagic acid (15 mg/kg) + isoproterenol (100 mg/kg)	131.00 ± 8.02^{d}	111.42 ± 7.65^{d}	91.83 ± 7.47^{d}	381 ± 10.56^{d}

Each value is mean ± standard deviation for six rats 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).

Table 1 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 ($P<0.05$). Oral pretreatment of 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 ($P<0.05$) increase in the heart rate of the isoproterenol-induced rats. The oral pretreatment of ellagic acid reduced the heart rate dose dependently and 15 mg/kg produced a better effect than 7.5 mg/kg.

The level of cardiac troponin-I in the serum of isoproterenol-induced rats showed a significant ($P<0.05$) increase, when compared to the normal control rats. The isoproterenol-induced rats showed a significant ($P<0.05$) increase in the activities of creatine kinase and lactate dehydrogenase in the serum, when 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 ($P<0.05$) inhibited the release of troponin-I, creatine kinase and lactate dehydrogenase, in the serum of isoproterenol-induced rats (Table 2).

The levels of C-reactive protein in the serum and homocysteine in the plasma of the normal and experimental rats were determined. The isoproterenol-induced myocardial infarcted rats showed a significant ($P<0.05$) increase in the levels of serum C-reactive protein and plasma homocysteine, when compared to the normal control rats. The oral pretreatment with ellagic acid (7.5 mg/kg and 15 mg/kg) significantly ($P<0.05$) decreased the levels of serum C-reactive protein and plasma homocysteine, when compared to the isoproterenol-induced rats (Table 2).

Table 3 shows the effect of ellagic acid on the concentrations of thiobarbituric acid reactive substances and lipid hydro peroxides in the heart tissue homogenates of normal and isoproterenol-induced rats. In the isoproterenol-induced rats, the concentrations of thiobarbituric acid reactive substances and lipid hydro peroxides were significantly ($P<0.05$) increased in the heart tissue, when compared with those of the normal control rats. Oral pretreatment with ellagic acid (7.5 mg/kg and 15 mg/kg) significantly ($P<0.05$) decreased the concentration of both the thiobarbituric acid reactive substances and lipid hydro peroxides in the heart tissue homogenate of isoproterenol-induced rats.

The activities of enzymatic antioxidants (superoxide dismutase and catalase) in the normal and experimental rats are studied. The isoproterenol-induced myocardial infarcted rats exhibited a significant ($P<0.05$) decrease in the activities of enzymatic antioxidants in the heart as compared to the normal control rats. The oral pretreatment of ellagic acid counteracted the deleterious effect of the isoproterenol dose dependently, by increasing the activity of superoxide dismutase and catalase significantly ($P<0.05$) as compared to the rats which received isoproterenol alone (Table 3).

Table 4 shows the levels/activities of reduced glutathione in the plasma and heart tissue homogenates, and glutathione dependant enzymatic antioxidants such as glutathione peroxidase, glutathione reductase, glutathione-s-transferase in the heart tissue homogenates of the normal and isoproterenol-induced rats. The isoproterenol-induced rats exhibited a significant ($P<0.05$) decrease in the levels of reduced glutathione, and activities of reduced glutathione dependant enzymatic antioxidants, when 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, when compared with the isoproterenol-alone-induced rats.

Rats induced with isoproterenol exhibited a significant ($P<0.05$) decrease in the levels of α-tocopherol and ascorbic acid in the plasma, when compared to the normal control rats. The oral pretreatment with ellagic acid (7.5 and 15 mg/kg) to the isoproterenol-induced rats significantly ($P<0.05$) increased the levels of α-tocopherol and ascorbic acid in the plasma, when compared to the isoproterenol-alone-induced rats (Table 5).

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 effect indicating that these doses appear safe.

4. Discussion

Increased generation of cytotoxic free radicals by auto-oxidation of isoproterenol subsequently damaged 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-

Table 2
Effect of ellagic acid on cardiac markers.

Groups	Serum troponin-I (ng/ml)	Serum creatine kinase (IU/L)	Serum lactate dehydrogenase (IU/L)	Serum C-reactive protein (mg/L)	Plasma homocysteine (ng/dL)
Normal control	0.22 ± 0.015^{a}	18.64 ± 1.74^{a}	84.29 ± 7.61^{a}	1.13 ± 0.09^{a}	3.90 ± 0.25^{a}
Normal + ellagic acid (7.5 mg/kg)	0.22 ± 0.019^{a}	19.24 ± 1.72^{a}	79.56 ± 7.16^{a}	1.12 ± 0.09^{a}	3.61 ± 0.36^{a}
Normal + ellagic acid (15 mg/kg)	0.22 ± 0.015^{a}	19.16 ± 1.86^{a}	77.51 ± 6.74^{a}	1.12 ± 0.09^{a}	3.55 ± 0.48^{a}
Isoproterenol-alone (100 mg/kg)	0.73 ± 0.06^{b}	36.75 ± 3.41^{b}	163.39 ± 17.56^{b}	8.17 ± 0.76^{b}	10.33 ± 1.11^{b}
Ellagic acid (7.5 mg/kg) + isoproterenol (100 mg/kg)	0.50 ± 0.035^{c}	26.80 ± 1.83^{c}	137.53 ± 9.11^{c}	4.83 ± 0.36^{c}	7.60 ± 0.90^{c}
Ellagic acid (15 mg/kg) + isoproterenol (100 mg/kg)	0.33 ± 0.032^{d}	23.52 ± 1.85^{d}	101.60 ± 8.04^{d}	2.82 ± 0.29^{d}	4.65 ± 0.46^{d}

Each value is mean ± standard deviation for six rats 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).

Table 3
Effect of ellagic acid on lipid peroxidation products and antiperoxidative enzymes in the heart tissue.

Groups	Thiobarbituric acid reactive substances (mmol/100 g wet tissue)	Lipid hydro peroxides (mmol/100 g wet tissue)	Superoxide dismutase (units/mg protein)	Catalase (μmol of hydrogen peroxide consumed/min/mg protein)
Normal control	0.67 ± 0.05^{a}	18.13 ± 1.79^{a}	9.34 ± 0.85^{a}	7.14 ± 0.47^{a}
Normal + ellagic acid (7.5 mg/kg)	0.68 ± 0.04^{a}	17.97 ± 1.70^{a}	9.76 ± 0.64^{a}	7.22 ± 0.46^{a}
Normal + ellagic acid (15 mg/kg)	0.68 ± 0.04^{a}	17.03 ± 1.43^{a}	9.86 ± 0.68^{a}	7.37 ± 0.59^{a}
Isoproterenol-alone (100 mg/kg)	1.01 ± 0.01^{b}	29.10 ± 2.77^{b}	4.59 ± 0.44^{b}	3.78 ± 0.40^{b}
Ellagic acid (7.5 mg/kg) + isoproterenol (100 mg/kg)	0.84 ± 0.06^{c}	23.74 ± 2.29^{c}	5.81 ± 0.47^{c}	4.84 ± 0.46^{c}
Ellagic acid (15 mg/kg) + isoproterenol (100 mg/kg)	0.72 ± 0.04^{d}	19.70 ± 1.74^{d}	8.21 ± 0.59^{d}	6.54 ± 0.52^{d}

Superoxide dismutase units: one unit is defined as the enzyme concentration required inhibiting the optical density at 560 nm of chromogen production by 50% in 1 min. Each value is mean ± standard deviation for six rats 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).

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 of ellagic acid significantly ($P<0.05$) 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 cardio protective effect of ellagic acid against isoproterenol-induced myocardial infarction.

Chronic stimulation of isoproterenol down regulated the β_1 and β_2 receptors (Bristow, 2000) and reduced arterial pressures in isoproterenol-induced rats. The supramaximal dose of isoproterenol also produced increased heart rate (tachycardia) due to involvement of baroreceptor reflex-mediated changes in autonomic nerve activity (Whalen and Lewis, 1999).

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), the oral pretreatment of ellagic acid (7.5 and 15 mg/kg) bind with them and significantly improves the arterial blood pressures and controlled the increased heart rate in the isoproterenol-induced rats.

Increased levels of cardiac markers such as cardiac troponin-I, creatine kinase and lactate dehydrogenase were observed in the isoproterenol-induced rats. Ellagic acid pretreatment at doses of 7.5 and 15 mg/kg in isoproterenol induced rats significantly ($P<0.05$) decreased the levels of these markers in the serum indicating its protective effect on the myocardium by reducing the extent of the myocardial damage, thereby restricting the leakage of these enzymes from the myocardium.

The increased level of C-reactive protein in the serum of isoproterenol-induced myocardial infarcted rats reflects extend of myocardial necrosis. The oral pretreatment of ellagic acid (7.5 and 15 mg/kg) significantly ($P<0.05$) reduced the elevated levels of C-reactive protein in the isoproterenol-induced rats. A recent study by Corbett et al. (2010), reported the anti-inflammatory effect of ellagic acid and its interaction with known cyclo oxygenase inhibitors. This known potential mechanism of ellagic acid is dependable with our results.

Homocysteine a product of methionine metabolism, a strong and independent risk factor for cardiovascular disease was significantly ($P<0.05$) increased in the plasma of the isoproterenol-induced rats, when 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. 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 increased level of inflammatory marker, C-reactive protein. The oral pretreatment of ellagic acid is able to inhibit the level of homocysteine in the isoproterenol-induced rats. This effect revealed the anti-inflammatory property of ellagic acid.

The degree of lipid peroxidation in the normal and experimental rats was assessed by estimating thiobarbituric acid reactive substances and lipid hydro peroxides. We observed an increase in the levels of thiobarbituric acid reactive substances and lipid hydro peroxides in the heart tissue of isoproterenol-induced rats. An enormous amount of superoxide radicals formed by isoproterenol can stimulate the Haber–Weiss reaction for further generation of reactive oxygen species, initiating lipid peroxidation (Becker, 2004). Pretreatment with ellagic acid significantly reduced the levels of thiobarbituric acid reactive substances and lipid hydro peroxides in the heart tissue. This effect showed the antilipid peroxidation property of ellagic acid. Previous studies also indicated that ellagic acid had a strong antioxidant effect (Festa et al., 2001), and inhibited lipid peroxidation (Osawa et al., 1987).

In the present study, we found decreased activities of anti peroxidative enzymes superoxide dismutase and catalase in the

Table 4
Efect of ellagic acid on reduced glutathione and glutathione dependant enzymatic antioxidants.

Groups	Glutathione in serum (mg/dL)	Glutathione in heart (mmol/gm wet tissue)	Glutathione peroxidase (μg of glutathione consumed/min/mg protein)	Glutathione reductase (Units/mg protein)	Gltathione-s-transferase (nmol of 1-chloro-2, 4-dinitrobenzene -glutathione conjugate formed/min/mg protein)
Normal control	24.96 ± 2.37^{a}	6.49 ± 0.44^{a}	4.78 ± 0.37^{a}	31.69 ± 2.94^{a}	0.95 ± 0.09^{a}
Normal + ellagic acid (7.5 mg/kg)	25.03 ± 2.31^{a}	6.67 ± 0.36^{a}	4.73 ± 0.36^{a}	32.32 ± 3.07^{a}	0.98 ± 0.09^{a}
Normal + ellagic acid (15 mg/kg)	25.21 ± 2.46^{a}	6.67 ± 0.32^{a}	4.75 ± 0.31^{a}	32.33 ± 3.19^{a}	0.96 ± 0.08^{a}
Isoproterenol-alone (100 mg/kg)	15.96 ± 1.28^{b}	3.14 ± 0.27^{b}	2.52 ± 0.25^{b}	15.71 ± 1.42^{b}	0.36 ± 0.04^{b}
Ellagic acid (7.5 mg/kg) + isoproterenol(100 mg/kg)	18.60 ± 1.13^{c}	4.45 ± 0.33^{c}	2.97 ± 0.25^{c}	22.53 ± 1.86^{c}	0.56 ± 0.05^{c}
Ellagic acid (15 mg/kg) + isoproterenol (100 mg/kg)	22.44 ± 2.05^{d}	5.17 ± 0.33^{d}	4.60 ± 0.39^{d}	26.28 ± 2.02^{d}	0.91 ± 0.09^{d}

Glutathione reductase units: μmol of NADPH oxidized/h/mg protein, each value is mean ± standard deviation for six rats 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).

Table 5
Effect of ellagic acid on α-tocopherol and ascorbic acid in plasma.

Groups	α-tocopherol (mg/dL)	Vitamin-C (mg/dL)
Normal control	2.25 ± 0.16^{a}	2.31 ± 0.18^{a}
Normal + ellagic acid (7.5 mg/kg)	2.22 ± 0.18^{a}	2.38 ± 0.23^{a}
Normal + ellagic acid (15 mg/kg)	2.18 ± 0.18^{a}	2.43 ± 0.22^{a}
Isoproterenol-alone (100 mg/kg)	0.63 ± 0.05^{b}	0.99 ± 0.08^{b}
Ellagic acid (7.5 mg/kg) + isoproterenol (100 mg/kg)	$1.09 + 0.09^{c}$	1.64 ± 0.12^{c}
Ellagic acid (15 mg/kg) + isoproterenol (100 mg/kg)	1.85 ± 0.09^{d}	2.20 ± 0.21^{a}

Each value is mean ± standard deviation for six rats 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).

isoproterenol-induced rats. These enzymes are utilized for scavenging superoxides and hydrogen peroxides which are produced by excessive dose of isoproterenol. The levels of reduced glutathione and the activities of glutathione peroxidase, and glutathione-s-transferase and glutathione reductase, in the isoproterenol-induced myocardial infarcted rats were significantly decreased, when compared to the normal control rats. The reduced glutathione levels might be due to its increased consumption by myocardium, in protecting thiol containing proteins from lipid peroxides. The unavailability of reduced glutathione may decrease the activities of glutathione peroxidase, glutathione-s-transferase and glutathione reductase in the isoproterenol-induced myocardial infarcted rats (Rajadurai and Stanely Mainzen Prince, 2006). The inactivation of glutathione reductase in the heart leads to the accumulation of oxidized glutathione. (Padmanabhan and Stanely Mainzen Prince, 2006). The decreased activity of glutathione-s-transferase reduces the metabolism of the xenobiotic isoproterenol and enhances the concentration in the circulatory system that lead to toxicity. The oral pretreatment of ellagic acid increases the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase and the levels of reduced glutathione in the heart tissue homogenate of the isoproterenol-induced rats. These pharmacological actions of ellagic acid could considerably develop cellular anti-oxidative defense against oxidative stress and protect myocardial tissues.

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

The histological study of the isoproterenol-induced myocardium showed large infarcted zones with edema, necrosis and separation of cardiac muscle fibers. The neutrophils characteristically invade the myocardial tissue during ischemia. The oral pretreatment of ellagic acid (7.5 and 15 mg/kg) showed protective effects in isoproterenol-induced cardio toxicity as evidenced by reduced necrosis and characteristic invasion of neutrophils compared to isoproterenol-induced rats heart. The histological examinations of the heart tissue of normal rats that received ellagic acid (7.5 and 15 mg/kg) alone did not show any significant changes in the myocardium indicating the non-toxic nature of ellagic acid.

A previous scientific report has also shown that caffeic acid, a phenolic compound exhibits protective effects on electrocardiogram (Senthil Kumaran and Stanely Mainzen Prince, 2010a), cardiac markers, lipid peroxidation, antioxidant system and histology of heart in isoproterenol induced myocardial infarction. (Senthil Kumaran and Stanely Mainzen Prince, 2010b).

In conclusion, the present study provides experimental evidence that the oral pretreatment of ellagic acid (7.5 and 15 mg/kg) was safe and highly effective in cardio protection, and improved cardiac dysfunction. These findings are rational to understand the beneficial effects of ellagic acid on cardio protection against myocardial injury, in which oxidative stress was known to contribute to the pathogenesis. Further research with the aim of determining the effects of ellagic acid on oxidative stress-induced myocardial infarction should include in clinical trials.

Acknowledgement

The author M. Mari Kannan, gratefully acknowledges the constant support and encouragement given by T.V.R. N. Reddy, Joint Secretary, Jayamukhi Educational Society, Narsampet, Warangal, Andhra Pradesh.

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