Protective efficacy of ellagic acid on glycoproteins, blood hematology, biochemical changes and electrolytes in myocardial infarcted rats

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ABSTRACT

The cardioprotective property of ellagic acid in rats has been reported previously. The present study reveals the protective role of ellagic acid in biochemical parameters including serum iron, plasma iron binding capacity, uric acid, glycoprotein and electrolytes along with haematological parameters. Rats were subcutaneously injected with isoproterenol (100mg/kg) for 2 days to induce myocardial infarction. Isoproterenol-induced rats showed a significant increase in their levels of serum iron, serum uric acid and blood glucose and a significant decrease in their levels of plasma iron binding capacity, serum total protein, albumin globulin ratio and heart glycogen, when compared with normal control rats. The altered haematological parameters were also observed in isoproterenol-induced rats when compared with normal control rats. Pretreatment with ellagic acid at doses of 7.5 and 15 mg/kg produced significant beneficial effect by returning all the above mentioned biochemical and haematological parameters to near normal levels.

INTRODUCTION

Many epidemiological studies have shown that myocardial infarction (MI) is one of the greatest risk factors for morbidity and mortality. A considerable number of MI cases are asymptomatic or associated with minor and atypical symptoms, and are found unintentionally during routine check ups that reveal the existence of risk factors. Changes in serum glycoprotein levels are characteristic of many pathological conditions. Morphological changes in the necrotic cell surface may alter the pattern of glycoprotein synthesis. The appearance of an abnormal level of different proteins in the blood reflects myocyte damage and helps to identify myocardial necrosis [1]. Shifts in electrolyte balance have been considered to play an important role in cardiac death and may be brought about by β adrenergic receptor stimulation. Examination of the blood can provide an early clue of cardiovascular health risk and may serve as a useful tool for monitoring the biological system.

Stimulation of β-adrenergic receptors by catecholoamines could be the triggering event for ventricular fibrillation and sudden cardiac death. Isoproterenol (ISO), a sympathomimetic beta adrenergic receptor agonist, responsible for the development of irreversible damage of myocardium in experimental animals and widely used in preclinical models for its extraordinary technical simplicity, excellent reproducibility and an acceptably low morbidity [2]. Several mechanisms of cardiotoxicity of ISO have been suggested including intracellular Ca^{2+} overload, changes in electrolytes content, oxidative stress, functional hypoxia and ischaemia.

Although the importance of diet and consumption of natural phenolic compounds in the prevention of heart disease may well be known among healthcare providers and the public, implementation of this knowledge has been limited. Preclinical and clinical studies fill these

critical gaps by providing a state-of-the-art compendium of the scientific evidence on the efficacy of coronary disease prevention using natural products. Ellagic acid, a naturally occurring polyphenolic compound found in different fruits like pomegranates, red raspberries, strawberries and blueberries and nuts such as walnut [3]. It has received particular attention because of its wide array of biological properties. The benefits of antioxidant, chemoprotective and hepatoprotective actions of ellagic acid have been proved in the past [4-6]. Recent studies from our laboratory showed ellagic acid exhibited cardioprotective activity and its membrane stabilizing effect on lysosomes and membrane bound ATPases [7, 8]. In continuation of our research on the effect of ellegic acid on ISO-induced MI, we studied the role of ellagic acid on glycoprotein contents, serum iron, iron binding capacity, uric acid, total protein, albumin globulin ratio, blood glucose, glycogen, electrolytes and haematological parameters.

MATERIALS AND METHODS

Animals

Wistar male albino rats weighing between 180–200 g were obtained from Srivenkateshwara Enterprises, Bangalore, Karnataka, India. The rats were housed in polypropylene cages lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22 °C. The rats were maintained on a commercial standard rat pellet diet and water ad libitum. All experimental protocols were approved by the Institutional Animal Ethical Committee, Jayamukhi College of Pharmacy, Narsampet, Warangal, Andhra Pradesh and experiments were performed according to the ethical guidelines of the Committee for the Purpose of Control and Safety of Experimental Animals (CPCSEA), New Delhi.

Drugs and Chemicals

Ellagic acid, isoproterenol, thiobarbituric acid and acetyl acetone reagent were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2, 2' dipyridyl, Sodium sulphite and Folin's phenol reagent were purchased from Himedia, Mumbai, India. All other chemicals used in the study were of the highest analytical grade.

Induction of experimental myocardial infarction

ISO (100 mg/kg body weight) was dissolved in saline and injected subcutaneously to rats daily for 2 consecutive days at an interval of 24 h [9].

Experimental Design

The animals were 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) dissolved 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) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group IV: normal control rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days and then subcutaneously injected with ISO (100 mg/kg) in 2 ml of saline at an interval of 24 h for 2 days (on 11^{th} and 12^{th} 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 ISO (100 mg/kg) at an interval of 24 h for 2 days (on 11^{th} and 12^{th} 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 ISO (100 mg/kg) once a day for 2 days (on 11^{th} and 12^{th} day). Twenty four hours after the second dose of ISO, the rats of all the groups were anesthetized and sacrificed by cervical decapitation. Blood was collected in two tubes, i.e., one with anticoagulant for haematological assays, and another without anticoagulant for serum separation. The serum was used for the estimation of biochemical parameters. The heart tissue was excised immediately and rinsed in ice-chilled saline. Internal coagulated blood was removed, and the heart was weighed to the nearest 0.1 g. Known weights of the tissues were weighed and homogenized in 5 ml of chilled 0.1 M tris-hydrochloric acid buffer (pH:7.4). The homogenate was centrifuged (3,000 ×g for 5 min) and the supernatant was used for the estimation of various biochemical parameters.

Estimation of biochemical parameters

Glycoprotein components such as hexose, hexosamine, fucose and sialic acid in the serum and heart tissue homogenate were estimated by the methods of Dubois and Gilles [10], Wagner [11], Dische and Shettle [12] and Warren [13] respectively.

Serum iron was determined by the method of Ramsay [14]. Plasma iron binding capacity was determined by the method of Ramsay [15]. Serum uric acid, serum total proteins, A/G ratio and blood glucose were estimated using reagent kits purchased from Randox Laboratories Limited, UK. The glycogen content in the heart was estimated by the method of Morales *et al* [16]. The protein content in the heart tissue homogenate was determined by the method of Lowry *et al.* [17].

Estimation of electrolyte

The concentration of Na^+ and K^+ ions in heart homogenate was estimated using commercial kits purchased from Monozyme India Limited, India. The level of Ca^{2+} ion in the heart was measured by the O-cresolphthalein complexone (OCPC) method using a reagent kit purchaged from Span Diagnostic Limited, India.

Haematology

Haematological parameters such as RBCs, Hb, PCV, WBCs, neutrophils, lymphocytes and eosinophils were analyzed in Auto Haematological Analyzer BC-3000 Plus (Mindray Company, Limited., China). Erythrocyte sedimentation rate was measured by the method of Bottiger and Svedberg [18]. A total fibrinogen level in the blood was measured by the method of Lempert [19].

Statistical analysis

Results are expressed as mean ± S.D. One way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Duncan's multiple range test (DMRT) using a Statistical Package for the Social Sciences (SPSS) software version 16.00, SPSS Inc., Chicago, IL, USA. 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.

RESULTS

The ISO-induced rats showed significant increase in the levels of glycoprotein components such as hexose, hexosamine, fucose and sialic acid in the serum (Fig. 1) and heart tissue homogenate (Fig. 2), when compared to the normal control rats. Pretreatment with ellagic acid (7.5 and 15 mg/kg) for 10 days, significantly decreased the levels of these glycoprotein components in the serum and heart tissue homogenate, when compared with ISO-induced non treated rats.

Table 1 illustrates the levels of serum iron, plasma iron binding capacity, uric acid, total protein, A/G ratio, glucose level in the blood and heart glycogen in normal and ISO-induced rats. ISO-induced rats showed a significant increase in the levels of serum iron, serum uric acid and blood glucose and a significant decrease in the levels of plasma iron binding capacity, serum total protein, A/G ratio and heart glycogen when compared with normal control rats. Pretreatment with ellagic acid (7.5 and 15mg/kg)daily for 10 days significantly decreased the levels in serum iron, serum uric acid and blood glucose and significantly increased the levels of plasma iron binding capacity, serum total protein, A/G ratio and heart glycogen when compared with ISO-induced non treated rats.

Table 2 shows the levels of Na^+, Ca^{2+} and K^+ ions in heart of normal and ISO-treated rats. The levels of Na^+ and Ca^{2+} ions were increased significantly with subsequent decrease in the levels of K^+ ion in ISO-induced rats when compared to normal control rats. Pretreatment with ellagic acid (7.5 and 15mg/kg) daily for a period of 10 days significantly decreased the levels of Na^+ and Ca^{2+} ions and significantly increased the levels of K^+ ion in ISO-induced rats when compared to ISO-induced non treated rats.

Table 3 shows the haematological parameters of normal and ISO-treated rats. The levels of RBCs, Hb, PCV, WBCs, neutrophils, platelet count and fibrinogen levels were increased significantly with subsequent decrease in the levels of ESR, lymphocytes and eosinophil in ISO-treated rats when compared with normal control rats. Pretreatment with ellagic acid (7.5 and 15mg/kg) daily for a period of 10 days significantly decreased the levels of RBCs, Hb, PCV, WBCs, neutrophils, platelet count and fibrinogen levels and increased the levels of ESR, lymphocytes and eosinophils when compared with ISO-induced non treated rats.

For all the biochemical parameters studied, ellagic acid at a dose of 15 mg/kg showed a higher significant effect than the lower dose (7.5mg/kg), and the oral administration of ellagic acid (7.5 mg/kg and 15mg/kg) for a period of 10 days in normal control rats had no appreciable effect.

DISCUSSION

Based on our previous report [8], EC_{50} value of ellagic acid is 28.5 μM as DPPH free radical scavenger. Previous studies on ellagic acid [20, 21] indicate lower bio availability of oral administration. Thus we selected higher doses such as 7.5mg/kg and 15mg/kg to achieve a better free drug concentration in systemic circulation.

Increased level of glycoprotein components is related to the severity and existence of degenerative vascular diseases [22]. A significant increase in glycoprotein levels in the serum and heart of the ISO-induced rats might be due to the secretion from cell membrane glycol-conjugates into the circulation which is a physiological adjustment to a pathological process [23]. Pretreatment with ellagic acid resulted in decreased levels of glycoprotein components and providing evidence on the membrane stabilizing effect of ellagic acid.

In hypoxic tissue, ATP depletion occurs, which leads to an accumulation of hypoxanthine and increased levels of xanthine oxidase [24]. This is a possible mechanism of ISO-induced hyperuricemia in myocardial infarcted rats. Ellagic acid, a phenolic acid can reduce hypoxia by regulating the cardiac rhythm and reducing oxidative damage [7].

A decrease in serum total proteins and A/G ratio could be due to increased free radical production by the administration of ISO. The ability of ellagic acid to scavenge free radicals and inhibit lipid peroxidation may help to increase serum protein level and A/G ratio in ISO-induced myocardial infarcted rats.

The changes in the levels of sodium and potassium in ISO-induced rats may be attributable to loss of cellular integrity, inhibition of the Na^+/K^+ adenosine triphosphatase function as a result of energy depletion and changes in the ratio of intracellular-to-extracellular

volume [25]. The level of glycoproteins in serum and tissues indicates the severity of degenerative cardiac disease [26]. The secretion of glycol conjugates from cell membranes into the circulation during cardiotoxicity may be the reason for increased glycoprotein levels in ISO-induced rats [27]. Pretreatment with ellagic acid regulated the electrolyte levels and decreased glycoprotein components in ISO-induced myocardial infarcted rats.

The observed increase in leucocytes and neutrophils in ISO-induced rats may be due to the inflammatory response to the ISO-induced damage in the myocardium. Pretreatment with ellagic acid increased the T-lymphocyte count in ISO-induced myocardial infarcted rats.

It is possible that polycythemia with released iron can cause endothelial dysfunction and thrombosis formation and both can alter rheological properties and vascular resistance with resultant tissue ischaemia [28]. Higher concentration of haemoglobin in ISO-induced rats may lead to increased oxygen delivery to tissues and generate oxygen free radicals, which can be harmful to the myocardium and other parts of the cardiovascular system. The antioxidant nature of ellagic acid can protect the myocardium from free radical damage.

The observed increase in fibrinogen level, platelet count and decreased level of ESR in ISO-induced rats is in close agreement with the clinical scenario in which enhanced platelet aggregation has been observed in patients with recent MI [29]. Pretreatment with ellagic acid reversed all the pathological changes and protected heart from ISO-induced myocardial infarction in rats.

Based on the results of this study, ellagic acid seems to be one of the promising molecules to explore therapeutic alternatives in the prevention of MI. Further clinical trials are necessary before ellagic acid could be developed as a drug for the treatment of MI in human.

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Figure legends

Fig. 1. Effect of ellagic acid on hexose, hexosamine, fucose and sialic acid in the serum of normal and experimental animals.

Each column is mean ± S.D. for six rats in each group; Columns not sharing a common letter (a, b, c, d) differ significantly with each other ($p < 0.05$, DMRT); Normal control group is compared with ellagic acid (7.5 and 15 mg/kg). Isoproterenol-induced (100mg/kg) group is compared with normal control group; Ellagic acid (7.5 mg/kg) + isoproterenol group and ellagic acid (15mg/kg) + isoproterenol group are compared with isoproterenol (100mg/kg).

Fig. 2. Effect of ellagic acid on hexose, hexosamine, fucose and sialic acid in the heart tissue homogenate of normal and experimental animals.

Each column is mean ± S.D. for six rats in each group; Columns not sharing a common letter (a, b, c, d) differ significantly with each other ($p < 0.05$, DMRT); Normal control group is compared with ellagic acid (7.5 and 15 mg/kg). Isoproterenol-induced (100mg/kg) group is compared with normal control group; Ellagic acid (7.5 mg/kg) + isoproterenol group and ellagic acid (15mg/kg) + isoproterenol group are compared with isoproterenol (100mg/kg).

Table 1: Effect of ellagic acid on biochemical parameters

Groups	**Serum iron** (µg/dL)	**Plasma iron binding capacity** (µg/dL)	**Serum uric acid** (mg/dL)	**Serum total proteins** (g/dL)	**A/G ratio**	**Blood glucose** (mg/dL)	**Glycogen** (mg/gm tissue)
Normal control	43.64±3.28[a]	29.43 ± 2.54[a]	1.43±0.13[a]	5.85±0.48[a]	2.85±0.34[a]	58.54 ± 3.75[a]	26.36±2.26[a]
Normal + EA (7.5 mg/ kg)	42.55±3.42[a]	29.65±2.65[a]	1.46±0.13[a]	5.74±0.47[a]	2.92±0.28[a]	56.63 ± 3.69[a]	25.45±2.12[a]
Normal + EA (15 mg/ kg)	41.34±3.53[a]	28.54 ±2.23[a]	1.38±0.11[a]	5.86±0.43[a]	2.78±0.25[a]	55.42 ± 3.87[a]	25.83±2.31[a]
ISO-alone	78.59±6.23[b]	14.56±1.22[b]	2.64±0.16[b]	3.83±0.24[b]	1.09±0.11[b]	89.76 ± 6.94[b]	15.34±1.32[b]
EA (7.5 mg/ kg) + ISO	61.43±3.88[c]	19.75±0.20[c]	1.87±0.14[c]	4.32±0.32[c]	1.92±0.44[c]	69.54 ± 4.65[c]	19.57±1.53[c]
EA (15 mg/ kg)+ ISO	47.37±3.45[d]	25.65±2.11[d]	1.56±0.14[d]	5.36±0.43[d]	2.34±0.18[d]	60.06 ± 5.32[d]	23.84±2.23[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 2: Effect of ellagic acid on electrolytes

Groups	Na^+	Ca^{2+}	K^+
	(nmol /mg protein)		
Normal control	4.14 ± 0.32[a]	10.43 ± 0.83[a]	5.23 ± 0.53[a]
Normal + ellagic acid (7.5 mg/ kg)	4.02 ± 0.36[a]	10.69 ± 0.78[a]	5.38 ± 0.46[a]
Normal + ellagic acid (15 mg/kg)	3.94 ± 0.26[a]	9.97 ± 0.69[a]	5.39 ± 0.44[a]
ISO-alone	9.32 ± 0.53[b]	19.12 ± 1.22[b]	3.07 ± 0.27[b]
Ellagic acid (7.5 mg/kg) + ISO	7.45 ± 0.43[c]	15.42 ± 1.43[c]	3.71 ± 0.28[c]
Ellagic acid (15 mg/kg) + ISO	5.28± 0.36[d]	12.05 ± 0.97[d]	4.66± 0.32[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 haematology

Groups	Normal control	Normal + ellagic acid (7.5 mg/ kg)	Normal + ellagic acid (15 mg/ kg)	ISO (150 mg/kg)	Ellagic acid (7.5 mg/ kg)+ ISO	Ellagic acid (15 mg/ kg)+ ISO
RBCs (millions/ cu.mm^{-1})	6.45 ±0.43^{a}	6.32±0.46^{a}	6.12±0.51^{a}	12.52±0.96^{b}	9.02±0.83^{c}	7.98±0.65^{d}
ESR (mm/ hr)	11.31±0.85^{a}	11.67±0.94^{a}	11.82±0.83^{a}	5.73±0.43^{b}	8.23±0.73^{c}	10.37±0.88^{d}
Hb (g/dL)	11.02±0.79^{a}	10.76±0.78^{a}	10.95±0.83^{a}	17.83±1.23^{b}	14.38±1.21^{c}	16.11±1.22^{d}
PCV (%)	19.54±1.44^{a}	19.44±1.85^{a}	18.21±1.24^{a}	36.83±2.84^{b}	27.27±1.68^{c}	20.76±1.65^{d}
WBCs (cells cu.mm^{-1})	6893± 432^{a}	6789±412^{a}	6882 ±429^{a}	9785±542^{b}	8612±518^{c}	7456±426^{a}
Neutrophils (%)	59.24±3.22^{a}	60.37±3.65^{a}	59.45±3.24^{a}	73.37±4.24^{b}	65.50±4.04^{c}	64.62±3.85^{d}
Lymphocytes (%)	28.32±1.81^{a}	29.65±2.32^{a}	28.66±2.16^{a}	17.12±1.53^{b}	20.45±1.77^{c}	24.62±1.82^{d}
Eosinophils (%)	3.65±0.28^{a}	3.43±0.31^{a}	3.56±0.36^{a}	1.82±0.15	2.34±0.26^{c}	3.12±0.26^{d}
Platelet count (10^{5}cells/cu.mm^{-})	1.55±0.12^{a}	1.53±0.09^{a}	1.64±0.18^{a}	3.14±0.22^{b}	2.62 ±0.21^{c}	1.87±0.12^{a}
Fibrinogen (mg/dL)	144.98± 6.55^{a}	139.54± 5.64^{a}	137.42±5.32^{a}	232.00±14.76^{b}	176.80±12.53^{c}	152.98±7.54^{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).