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Original article

Protective effect of rutin and ellagic acid on isoproterenol-induced myocardial infarction in wistar rats

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Abstract:

The present study investigated the protective effect of rutin and ellagic acid (EA) alone and in combination on serum and heart tissue lipids and membrane bound enzymes on isoproterenol (ISO)-induced myocardial infarction (MI) in rats. Rats induced with ISO (85 mg/ kg) showed a significant increase in the level of total cholesterol (TC), triglyceride (TG) and free fatty acid (FFA) in serum and heart and altered levels of lipoproteins in serum were observed. Phospholipids (PL) level were increased in serum and decreased in heart in ISO-induced rats. Altered activities of membrane bound enzymes and the levels of glucose, uric acid and protein were observed in ISO-induced rat heart. Pretreatment with rutin (10 mg/kg) and EA (30 mg/kg) alone and in combination for a period of 14 days exhibited a significant protective effect on lipids, lipoproteins, membrane bound enzymes and other biochemical parameters in ISO- induced rats. Thus our findings indicate that rutin and EA have cardioprotective effect in ISO-induced MI in rats. Also found that combined treatment possesses more protection than individual treatment.

1. INTRODUCTION

Cardiovascular disease (CVD) is the principal cause of death, accounting around 20% of annual deaths worldwide. CVD is a heterogeneous group of disorders that affect the heart and blood vessels. Myocardial infarction (MI) is one of the main causes of death from CVDs (Padmanabhan & Prince, 2006). MI is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Despite all basic and clinical improvements, MI is still one of the most common and severe health problems in the modern world (Aronow, 2006).

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, causes severe stress in the myocardium resulting in infarct like necrosis on the heart muscles. ISO-induced MI is a well standardized model to study the beneficial effects of various drugs and cardiac function (Ithayarasi & Devi, 1997). It is also known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane (Kakreja & Hess, 1992). ISO-administration causes an increase in the levels of circulatory and myocardial lipids, indicating its hyperlipidemic effect (Chagoya de Sanchez et al., 1997). High levels of circulating cholesterol and its accumulation in cardiac tissue are well associated with cardiovascular damage. Thus the lipids play an important role in the pathogenesis of MI. An increase in the concentration of total and LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of MI (Mediene-Benchekor, 2001).

Flavonoids, a large class of phenolic compounds widely distributed in plants, fruits, vegetables, nuts and whole grains have been reported to be strong antioxidants and radical

scavengers (Papadopoulou, 2005). Rutin is a bioflavonoid glycoside known as Vitamin P, abundantly present in onions, apples, tea and red wine (Hertog, 1993). It includes multiple pharmacological activities such as antibacterial and antiviral (Panasiak et al., 1989), antiprotozoal (Iwu et al., 1986), antitumor (Deschner et al., 1991), antiallergic (Chen et al., 2000), anti-inflammatory (Aleksandrov et al., 1986) and antiplatelets (Swies et al., 1984), anti-diabetic properties (Kamalakkannan & Prince, 2006a, 2006b), anti-diarrheal, antiulcer, anti-mutagenic, vasodilator, immunomodulatory and hepatoprotective (Brenna & Pagliarini, 2001) properties. Ellagic acid (EA), a phenolic acid naturally occurring in many plant foods such as carrot, tomato, raspberries, cranberries, walnuts, pecans, pomegranates, strawberry and blueberry (Sellappan et al., 2002; Mattila & Kumpulainen, 2002]. It has been documented that the phenolic acids possess anti-oxidative activities such as scavenging free radicals and chelating metal ions (Makena & Chung, 2007; Prakash et al., 2007) antiviral and antibacterial properties (Xu et al., 2003).

According to medical practitioners, a combination of drugs exhibits augmented protective efficacy than a single drug. The available options for the pharmacotherapy of MI are still inadequate in reducing the high mortality and thus novel and effective therapeutic modalities are needed for the treatment of MI. The major purpose of this study was to investigate the combined effect of rutin and EA acid on membrane bound enzymes such as sodium/potassium dependent adenosine triphosphatase (Na+/K+-ATPase), calcium dependent adenosine triphosphatase (Ca2+-ATPase), magnesium dependent adenosine triphosphatase (Mg2+-ATPase) and lipids like triglyceride (TG), total cholesterol (TC), free fatty acid (FFA), phospholipids (PL), high density lipo protein (HDL), low density lipoprotein (LDL) and very low density lipo protein (VLDL) and also the levels of total protein, glucose and uric acid in ISO-induced MI in rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

All the experiments were done with female albino Wistar rats weighing 140-160 g, obtained from the Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22˚C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolisable energy of 3,600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.2 Drug and chemicals

Isoproterenol hydrochloride, rutin and EA were purchased from Sigma chemicals, St. Louis, MO, USA. Adenosine triphosphate (ATP), triethanolamine (TEA) copper nitrate and diphenyl carbazide were obtained from Himedia laboratory, Mumbai, India. Triglycerides (TG), HDL cholesterol, glucose, protein and uric acid kits were purchased from Agappe Diagnostics, Kerala, India.

Induction of Experimental Myocardial Infarction

Isoproterenol (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for two days (Gupta et al., 2004).

2.3 Experimental Design

A total of 36 rats were divided into 6 groups (6 rats in each group).

Group 1: Normal control rats
Group 2: Rutin (10 mg/kg) + EA (30 mg/kg)
Group 3: ISO control rats (85 mg/kg)
Group 4: Rutin (10 mg/kg) + ISO
Group 5: EA acid (30 mg/kg) + ISO
Group 6: Rutin (10 mg/kg) + EA (30 mg/kg) + ISO

EA and rutin were dissolved in 0.2% DMSO and double distilled water, respectively and administrated to rats orally using an intragastric tube daily for a period of 14 days. At the end of treatment period all the rats were injected with ISO (85 mg/kg) at an interval of 24 h for 2 days (on 15th and 16th day). Twelve hours after the second dose of ISO injection (on 17th day), all the rats were anesthetized with pentobarbital sodium (35 mg/kg) and sacrificed by cervical decapitation. Blood was collected; serum was separated and used for various biochemical estimations. The heart tissue was excised immediately from the rats, washed off blood with ice-chilled physiological saline and used for further biochemical estimations. A known weight of the heart tissue was homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

2.4 Biochemical Estimations

Lipids were extracted from serum and tissues by the method of Folch et al. (1957). Total cholesterol was estimated by the method of Zlatkis et al. (1953). HDL levels were estimated by

Assmann (1979) method using a commercial kit (Product No. 11010001) obtained from Agappe Diagnostics, Kerala, India. Triglyceride levels were estimated by Schettler (1975) method using a commercial kit (Product No. 11409001) obtained from Agappe Diagnostics, Kerala, India. Free fatty acids levels were estimated by the method of Falholt et al. (1973). Phospholipids levels were estimated by the method of Zilversmit et al. (1950). LDL-cholesterol and VLDL-cholesterol were calculated as follows:

VLDL = TG/5

LDL = Total cholesterol - (HDL + VLDL)

Glucose levels were estimated by Digeon (1975) method using a commercial kit (Product No. 11406001) obtained from Agappe Diagnostics, Kerala, India. Serum uric acid levels were estimated by Fossatai (1980) method using a commercial kit (Product No. 11413001) obtained from Agappe Diagnostics, Kerala, India. Total protein levels were estimated by Doumas (1971) method using a commercial kit (Product No. 11002001) obtained from Agappe Diagnostics, Kerala, India. The activity of Na+/K+-ATPase was assayed according to the procedure of Bonting et al. (1970). The activity of Ca2+-ATPase was assayed according to the method of Hjerten et al. (1983). The activity of Mg2+-ATPase was assayed by the method of Ohnishi et al. (1982).

2.5 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 Sciences (SPSS) software package version 9.05. P values <0.05 were considered significant.

3. RESULTS AND DISCUSSION

Administration of ISO caused a significant increase in the levels of TC, TG, PL and FFA in serum represented in Table 1. There was a significant increase in the levels of TC, TG and FFA and decreased in the levels of PL in heart of ISO-induced rats when compared to normal rat (Table 2). Administration of rutin (10 mg/kg) and EA (30 mg/kg) alone and in combination for a period of 14 days significantly decreased the level of TC, TG and FFA in serum and heart of ISO-induced rats. PL was decreased in serum and increased in heart of rutin and EA treated rats.

Rats treated with ISO, showed a significant increase in the levels of LDL and VLDL and decreased in the levels of HDL as comparer with normal rats. Pretreatment with rutin and EA showed a significant decrease in the level of LDL and VLDL with subsequent increase in the level of HDL represented in Figures 1 and 2.

Table 1. Effect of rutin and ellagic acid (EA) on the levels of total cholesterol (TC), triglyceride (TG), phospholipids (PL) and free fatty acid (FFA) in serum of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	TC (mg/dl)	TG (mg/dl)	PL (mg/dl)	FFA (mg/dl)
Normal control	113.8 ± 5.38^{a}	51.8 ± 3.31^{a}	53.4 ± 4.05^{a}	23.6 ± 1.25^{a}
Normal + rutin (10 mg/kg) + EA (30 mg/kg)	112.2 ± 6.12^{a}	50.6 ± 2.83^{a}	52.6 ± 3.77^{a}	23.4 ± 1.37^{a}
ISO (85 mg/kg) control	182.5 ± 8.17^{b}	78.3 ± 5.45^{b}	78.5 ± 5.15^{b}	59.7 ± 3.16^{b}
Rutin (10 mg/kg) + ISO	141.6 ± 7.23^{c}	63.8± 4.76^{c}	65.8 ± 4.18^{c}	39.2 ± 2.76^{c}
EA (30 mg/kg) + ISO	143.5 ± 5.84^{c}	64.9 ± 4.12^{c}	66.1 ± 3.63^{c}	38.6 ± 2.54^{c}
Rutin (10 mg/kg) + EA (30 mg/kg) + ISO	130.2 ± 6.65^{d}	58.7 ± 3.17^{d}	60.2 ± 3.29^{d}	27.3 ± 1.83^{d}

Table 2. Effect of rutin and ellagic acid (EA) on the levels of total cholesterol (TC), triglyceride (TG), phospholipids (PL) and free fatty acid (FFA) in heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	TC (mg/g wet tissue)	TG (mg/g wet tissue)	PL (mg/g wet tissue)	FFA (mg/g wet tissue)
Normal control	5.38 ± 0.21^{a}	2.35 ± 0.14^{a}	23.4 ± 1.44^{a}	0.27 ± 0.02^{a}
Normal + rutin (10 mg/kg) + EA (30 mg/kg)	5.27 ± 0.27^{a}	2.27 ± 0.19^{a}	23.3 ± 1.27^{a}	0.26 ± 0.01^{a}
ISO (85 mg/kg) control	9.13 ± 0.18^{c}	6.00 ± 0.36^{b}	12.4 ± 0.92^{b}	0.59 ± 0.02^{b}
Rutin (10 mg/kg) + ISO	7.70 ± 0.36^{b}	4.58 ± 0.24^{c}	17.9 ± 0.85^{c}	0.39 ± 0.02^{c}
EA (30 mg/kg) + ISO	7.66 ± 0.22^{c}	4.65 ± 0.21^{c}	18.1 ± 1.11^{c}	0.38 ± 0.03^{c}
Rutin (10 mg/kg) + EA (30 mg/kg) + ISO	6.50 ± 0.41^{d}	3.44 ± 0.20^{d}	20.6 ± 1.63^{d}	0.32 ± 0.01^{d}

Each value is mean ± S.D. for 6 rats in each group.

Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

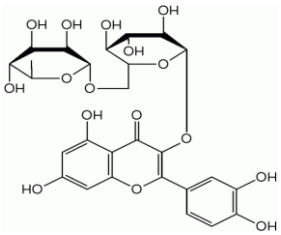


Figure 1. Structure of rutin

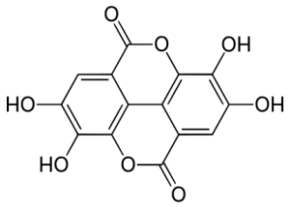


Figure 2. Structure of ellagic acid

Table 3 represents the levels of blood glucose, serum uric acid and total protein of normal and ISO-induced rats respectively. In ISO-induced rats, a significant increase in the levels of blood glucose and uric acid and decreased in the level of total protein were observed. Oral administration of rutin and EA significantly decreased the levels of blood glucose, uric acid and increased the level of total protein when compared with ISO control rats.

Table 3. Effect of rutin and ellagic acid (EA) on the levels of blood glucose, serum uric acid and total protein of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

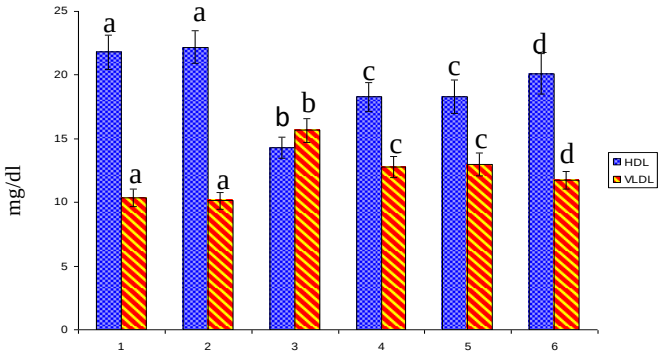
Groups	Glucose (mg/dl)	Uric acid (mg/dl)	Total protein(g/dl)
Normal control	97.4 ± 5.20[a]	1.88 ± 0.09[a]	6.84 ± 0.24[a]
Normal + rutin (10 mg/kg) + EA (30 mg/kg)	95.8 ± 4.86[a]	1.81 ± 0.10[a]	7.01 ± 0.27[a]
ISO (85 mg/kg) control	168.6 ± 9.15[b]	4.76 ± 0.26[b]	4.85 ± 0.18[b]
Rutin (10 mg/kg) + ISO	122.5 ± 8.13[c]	3.16 ± 0.23[c]	5.51 ± 0.20[c]
EA (30 mg/kg) + ISO	123.1 ± 6.44[c]	3.29 ± 0.17[c]	5.50 ± 0.31[c]
Rutin (10 mg/kg) + EA (30 mg/kg) + ISO	107.7 ± 6.63[d]	2.49 ± 0.13[d]	6.13 ± 0.26[d]

Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Figures 3 and 4 present the activities of membrane bound ATPases (Na+/K+-ATPase, Ca2+-ATPase and Mg2+-ATPase) in heart of normal and ISO-induced rats. The activity of Na+/K+-ATPases was significantly decreased and the activities of Ca2+-ATPases and Mg2+-ATPases were significantly increased in heart of ISO-induced rats. Oral pretreatment with rutin and EA increased the activity of Na+/K+-ATPases and decreased the activities of Ca2+-ATPases and Mg2+-ATPases in the heart of ISO-induced rats.

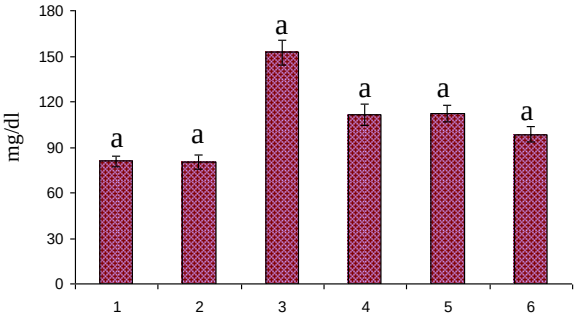
For all the parameters studied pretreatment with rutin (10 mg/kg) and EA (30 mg/kg) combination to normal rats for a period of 14 days did not show any significant effect. Pretreatment with rutin and EA alone and in combination to ISO-induced rats showed significant effects. Combined treatment with rutin and EA showed more pronounced effect than individual treatment.

ISO is a dual β1and β2-adrenergic receptor agonist that has acute positive chronotropic and inotropic effects on the heart muscles (Kitagawa et al., 2004). Among other mechanisms proposed to explain ISO-induced cardiac damage are its stimulation of adenylate cyclase and its activation of Na+ and Ca2+ channels (Nayira et al., 2009).



Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Figure 3. Effect of rutin and ellagic acid (EA) on the levels of high density lipoprotein (HDL) and very low density lipoprotein (VLDL) in serum of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.



Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Figure 4. Effect of rutin and ellagic acid (EA) on the levels of low density lipoprotein (LDL) in serum of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

This leads to exaggerated intracellular Ca2+ concentrations and consequently to an excessive activation of Ca2+-dependent intracellular ATPases. Loss of function and integrity of myocardial membranes are the outcomes of ISO-induced myocardial injury involving change in membrane permeability alterations (Thippeswamy et al., 2009).

Lipids play a vital role in CVD by the way of hyperlipidaemia, development of atherosclerosis and also by modifying the cellular membrane composition, structure and stability (Rajadurai & Prince, 2005). Administration of ISO raised the levels of total cholesterol, TG, PL, FFA, LDL and VLDL and decreased HDL levels in the serum. These changes could be due to enhanced lipid biosynthesis by cardiac cAMP. An increase in concentration of total cholesterol and LDL, and a decrease in

HDL are associated with raised risk of MI (Aronow, 2006). The biochemical changes on ISO-administration in particular, altered lipid metabolism is comparable to human. High levels of circulating cholesterol and its accumulation in cardiac tissue are well associated with cardiovascular damage. ISO induces free radical formation, which may cause cellular cholesterol accumulation by increasing cholesterol biosynthesis, by decreasing cholesteryl ester hydrolysis and by reducing cholesterol efflux (Deepa & Varalakshmi, 2005).

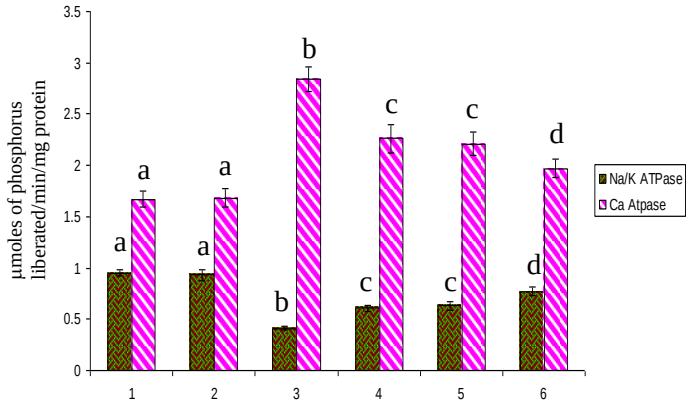
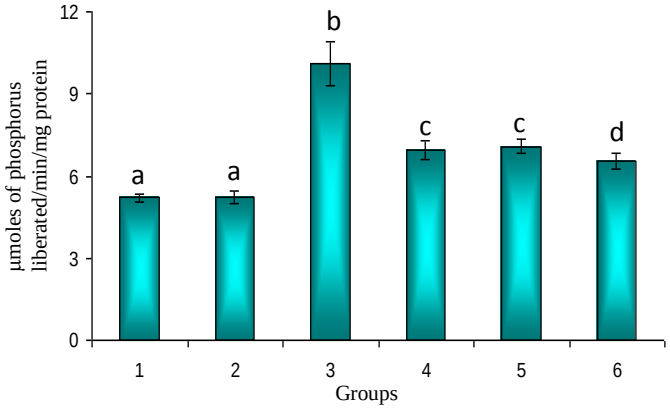


Figure 5. Effect of rutin and ellagic acid (EA) on the activities of, sodium/potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase) and calcium dependent adenosine triphosphatase (Ca^{2+}-ATPase) in heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.



Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Figure 6. Effect of rutin and ellagic acid (EA) on the activity of magnesium dependent adenosine triphosphatase (Mg^{2+}-ATPase) in heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Alterations in lipid composition observed in damaged myocardial tissue appear to occur due to destruction of cardiomyocytes (Borinski et al., 1993). An inverse relationship exists between HDL cholesterol and body cholesterol. HDL inhibits the uptake of LDL by the arterial wall and facilitates the transport of cholesterol from peripheral tissue to the liver, where it is catabolised and excreted from the body (Sheela & Devi, 2001). LDL could be oxidized and accumulated in macrophages, which turn into foam cells. Foam cells are the initial step in the formation of atherosclerotic plaques.

Combined treatment with rutin (10 mg/kg) and EA (30 mg/kg) alone and in combination for a period of 14 days showed a significant decrease in the level of serum and heart lipids and increased heart PL and serum HDL cholesterol in ISO-induced rats. This could be due to lipid lowering property of rutin and EA. It is reported that, rutin posses lipid lowering property in ISO-induced MI in rats (Prince & Sathy, 2010). EA has antioxidant property, which indirectly helps to decrease the levels of lipids by preventing the membrane damage. Many flavonoids have extensive biological properties that reduce the risk of heart disease. They protect LDL cholesterol from oxidation, inhibit the formation of blood clots and have hypolipidemic effects and anti-inflammatory action (Manach, 1996).

In this study, decreased levels of serum total proteins and increased level of blood glucose and uric acid were observed in ISO-induced rats. A decrease in serum total proteins could be due to increased free radical production by the administration of ISO. An increased in the level of blood glucose in ISO-induced rats is due to the enhanced glycogen breakdown and less utilization (Prabu et al., 2006). Increased level of serum uric acid considered to be a risk factor in MI (Weir et al., 2003). The conversion of xanthine dehydrogenase to xanthine oxidase occurs in ischaemic tissue (Mc Cord, 1988). Depletion of ATP occurs during hypoxia causes accumulation of hypoxanthine. Xanthine oxidase involved in the conversion of hypoxanthine to xanthine, uric acid and superoxide (Padmanabhan et al., 2008). This could be one of the reasons for the elevated levels of uric acid in ISO-induced rats. Pretreatment with rutin and EA significantly increased the level of serum protein and reduced the levels of blood glucose and serum uric acid in ISO-induced rats. It has been already reported that, rutin possess glucose lowering property in experimental study (Kamalakkannan & Prince, 2006a). Rutin and EA posses free radical scavenging and antioxidant properties, which could the reason for the minimizing the alterations in these biochemical parameters.

Membrane bound ATPase play an imperative role in the process of contraction and relaxation of the cardiac muscle by maintaining normal ion levels within the myocytes. Altered activities of these enzymes affect the role of heart. Factors like lipid peroxidation and membrane fluidity etc. can alter the activities of ATPase. Peroxidation of membrane lipids could inactivate Na+/K+-ATPase due to the oxidation of 'SH' groups present in its active site, leading to the conformational alterations of the enzyme (Kako et al., 1988). The decrease in the activity of Na+/K+-ATPase and increased activities of Ca2+ and Mg2+-ATPases observed in ISO-induced rats. The inhibition of Na+/K+-ATPase can turn on the Na+/Ca2+ exchange machinery in the myocardium. This Na+/K+ exchange mechanism may play a role in regulating the cellular calcium level (Trump et al., 1986). Oral pretreatment with rutin and EA

to ISO-induced rats increased the activity of Na+/K+-ATPase and decreased the activities of Ca2+ and Mg2+-ATPases in the heart. Increased Na+/K+-ATPase activity due to pretreatment of rutin and EA could regulate the intracellular Ca2+ levels, thereby protecting the myocardium from excess damage by maintaining the membrane reliability (Gubdjorson et al., 1983). These effects show membrane stabilizing property of rutin and EA.

On oral administration, rutin can be hydrolyzed by the intestinal microflora with α-rhamnosidase and β-glucosidase to isoquercitrin (quercetin 3-glucoside) and quercetin (Bokkenheuser et al., 1987). The formed quercetin is absorbed in the gastrointestinal tract and excreted through the bile and urine as glucuronide and sulfate conjugates (Ueno et al., 1983). Rutin have many of the structural components, which contribute to its antioxidant property. The presence of a hydroxyl groups, double bond between carbons two and three (C2-C3) and the carbonyl group at C-4 in its structure, which may form chelate with iron ions, which may contribute to their antilipoperoxidative properties by preventing the formation of free radicals. EA possesses antioxidant activity such as scavenging free radicals and increasing the activities of antioxidant enzymes (Makena & Chung, 2007). It controls the cardiac oxidative stress via reducing the formation and ROS and enhance antioxidant defense by increasing GSH retention and restoring the activity of antioxidant enzymes. Thus the rutin and EA scavenges superoxide radicals and hydrogen peroxide produced by ISO and reduces myocardial damage (Punithavathi et al., 2010).

Conclusion

Rutin and EA pretreatment significantly minimized the alterations in all the biochemical parameters. The pharmacological properties like free radical scavenging, antioxidant, lipid lowering, membrane stabilizing and cytoprotective effect of rutin and EA may directly or indirectly helps to decrease the levels of lipids and lipopoteins and maintaining the activities of membrane bound enzymes in ISO-induced rats.

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