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Preventive effect of rutin, a bioflavonoid, on lipid peroxides and antioxidants in isoproterenol-induced myocardial infarction in rats

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Abstract

The consumption of diets rich in plant foods is associated with a reduced risk of cardiovascular diseases. This study aimed to evaluate the preventive role of rutin on lipid peroxides and antioxidants in normal and isoproterenol-induced myocardial infarction in rats. Subcutaneous injection of isoproterenol (150 mg kg^{-1}) to male Wistar rats at an interval of 24 h for two days showed a significant increase in the activity of serum cardiac marker enzymes (creatine kinase, lactate dehydrogenase, aspartate transaminase and alanine transaminase) and a significant decrease in the activity of these enzymes in the heart. Lipid peroxidative products (thiobarbituricacid reactive substances and lipid hydroperoxides) were significantly increased and enzymic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymic (reduced glutathione and vitamin C) antioxidants showed a significant decrease in isoproterenol-treated rats. Pretreatment with rutin (40 or 80 mg kg^{-1}) to isoproterenol-treated rats orally for a period of 42 days daily caused a significant effect. Administration of rutin to normal rats did not have any significant effect on any of the parameters studied. The results of our study show that rutin possesses antioxidant activity in isoproterenol-induced experimental myocardial infarction.

Introduction

Cardiovascular disease (CVD) remains the principal cause of death in both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year. Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (De Bono & Boon 1992). Loper et al (1991) reported that there is increased lipid peroxidation and a transient inhibition of protective enzymes, such as superoxide dismutase (SOD), in both MI and unstable angina.

Administration of isoproterenol, a β-adrenergic agonist and synthetic catecholamine, depletes the energy reserve of cardiac muscle cells and causes complex biochemical and structural changes leading to cell damage and necrosis (Rona 1985). Wexler & Greenberg (1978) reported that the pathophysiological changes following isoproterenol administration are comparable with those taking place in human MI. There has been increasing recognition that certain natural substances have the potential to reduce the detrimental effect of a number of cardiovascular risk factors.

Dietary factors play an important role in the development of various human diseases, including cardiovascular disease. Epidemiological studies have shown that diets rich in fruits, herbs and spices are associated with a low risk of cardiovascular disease (Banerjee & Maulik 2002). Flavonoids, plant-derived antioxidants, are defined as non-nutritive dietary components that are abundant in foods (Boyle et al 2000). Consumption of flavonoid-containing foods and beverages has been proposed as a useful practice to limit oxidative damage in the body (Cherubini et al 1999). The protective role of flavonoids involves several mechanisms of action: a direct antioxidant effect, inhibition of enzymes of the oxygen-reduction pathway, and sequestration of transient metal cations (Robak & Gryglewski 1996; Cotelle 2001; Rice-Evans 2001).

Rutin is abundantly present in onions, apples, tea and red wine (Hertog et al 1993). A wide variety of pharmacological actions of rutin were reported – antibacterial, anti-tumour,

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anti-inflammatory, anti-diarrhoeal, anti-ulcer, anti-mutagenic, vasodilator, immunomodulatory and hepatoprotective activity (Janbaz et al 2002). This study addresses the preventive effect of rutin on lipid peroxidation and antioxidants in isoproterenol-induced MI in rats.

Materials and Methods

Animals

All the experiments were carried out with male albino Wistar rats, 150–170 g (Central Animal House, Rajah Muthiah Institute of Health Sciences, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India). They were housed in polypropylene cages ($47 \times 34 \times 20$ cm) lined with husk, renewed every 24 h, under a 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 23% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorous, 3.4% glucose and 55% nitrogen-free extract (carbohydrates). The diet provided a metabolizable energy of 3000 kcal kg^{-1}. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Approval No. 262, dated 16.12.2004).

Chemicals

Rutin hydrate, isoproterenol hydrochloride, butylated hydroxy toluene, nitroblue tetrazolium, phenazine methosulfate and glutathione were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and biochemicals used were of high analytical grade.

Experimental design

A pilot study was conducted to determine the effect of rutin at three different doses (20, 40 and 80 mg kg^{-1}) for a period of 45 days in isoproterenol-induced rats. Since 40 and 80 mg kg^{-1} showed significant ($P < 0.05$) effects in isoproterenol-induced rats, we used 40 mg and 80 mg kg^{-1} of rutin for further studies. Rutin at a dose of 20 mg kg^{-1} did not show any significant effect in isoproterenol-induced rats.

The rats were divided into 6 groups of 6 rats as follows: group 1, normal untreated rats; group 2, normal rats orally treated with rutin 40 mg kg^{-1} using an intragastric tube daily for 42 days; group 3, normal rats treated orally with rutin 80 mg kg^{-1} using an intragastric tube daily for 42 days; group 4, rats subcutaneously injected with isoproterenol 150 mg kg^{-1} dissolved in saline, once a day for 2 days; group 5, rats preteated with rutin 40 mg kg^{-1} orally using an intragastric tube daily for 42 days and then subcutaneously injected with isoproterenol 150 mg kg^{-1} once a day for 2 days; group 6, rats pretreated with rutin 80 mg kg^{-1} orally using an intragastric tube daily for 42 days and then subcutaneously injected with isoproterenol 150 mg kg^{-1} once a day for 2 days. Rutin was suspended in carboxymethylcellulose (CMC) 0.01 g mL^{-1} and was orally administered to rats (1 mL/rat). Normal, control and isoproterenol-control rats received CMC alone (1 mL/rat).

Twenty-four hours after the second dose of isoproterenol, all the rats were sacrificed by cervical decapitation. Blood was collected in two different tubes (i.e. one with anticoagulant, for plasma separation, and another without anticoagulant, for serum separation). Plasma and serum were separated by centrifugation and used for various biochemical estimations.

The heart was dissected out, washed immediately in ice-chilled physiological saline, blotted and weighed. A known weight (200 mg) of the heart tissue was homogenized in 5 mL of 0.1 M Tris-HCl (pH 7.4) buffer solution. The homogenate was centrifuged at 3000 rpm for 5 min. The supernatant was used for the estimation of various biochemical parameters.

Biochemical estimations

Assay of creatine kinase

The activity of creatine kinase in serum and heart was assayed by the method of Okinaka et al (1961). The incubation mixture, containing 0.75 mL of double-distilled water, 0.05 mL of serum/0.5 mL of tissue homogenate, 0.1 mL of ATP solution, 0.1 mL of magnesium-cysteine reagent and 0.1 mL of creatine, was incubated at 37°C for 20 min. The tubes were centrifuged and the supernatant was used for the estimation of phosphorus by the Fiske & Subbarow (1925) method. One millilitre of the supernatant was made up to 4.3 mL with water and 1 mL of ammonium molybdate reagent was added and incubated at room temperature for 10 min. Then 0.4 mL of ANSA was added and the colour developed was read at 640 nm after 20 min.

Assay of lactate dehydrogenase (LDH)

Lactate dehydrogenase in serum and heart was assayed by the method of Buhl & Jackson (1978) using a reagent kit (Product No.72351, Qualigens diagnostics, Mumbai, India). To 0.1 mL of reagent (substrate, buffer and co-enzyme), 30 μL of sample was added, mixed well and incubated for 1 min at 37°C and the absorbance was measured at 340 nm. Blank and standards were also treated in a similar manner.

Assay of aspartate transaminase (AST)

Aspartate transaminase in serum and heart was assayed by the method of Reitman & Frankel (1957) using a reagent kit (Qualigens diagnostics, Product No. 72141, Mumbai, India). Buffered substrate (0.5 mL) was incubated at 37°C for 3 min and 0.1 mL of serum/0.1 mL of homogenate was added, mixed well and incubated at 37°C for 30 min. Then 0.5 mL of 2,4-dinitrophenyl hydrazine (DNPH) reagent was added, mixed well and kept at room temperature for 20 min and 0.5 mL of 4 N working sodium hydroxide was added, and kept at room temperature for 10 min. Blank and standards were also processed in a similar way and the absorbance was measured spectrophotometrically at 505 nm.

Assay of alanine transaminase (ALT)

Alanine transaminase in serum and heart was assayed by the method of Reitman & Frankel (1957) using a reagent kit

(Product No.72151, Qualigens diagnostics, Mumbai, India). Buffered substrate (0.5 mL) was incubated at 37°C for 3 min and 0.1 mL of serum/0.1 mL of homogenate was added, mixed well and incubated at 37°C for 60 min. Then 0.5 mL of DNPH reagent was added, mixed well and kept at room temperature for 20 min, after which 0.5 mL of 4 N working sodium hydroxide was added and kept at room temperature for 10 min. Blank and standards were also processed in a similar way and the absorbance was measured spectrophotometrically at 505 nm.

Estimation of plasma TBARS

Plasma thiobarbituric acid reactive substances (TBARS) was estimated by the method of Yagi (1987). To 0.5 mL of plasma, 4.0 mL of 0.83 N H_2SO_4 and 0.5 mL of 10% phosphotungstic acid were added and mixed. After standing for 5 min, the mixture was centrifuged at 3000 *g* for 10 min. The supernatant was discarded and the sediment was mixed with 2.0 mL of H_2SO_4 and 0.3 mL of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at 3200 rpm for 10 min. The sediment was suspended in 4.0 mL of distilled water and 1.0 mL of TBA reagent was added. The reaction mixture was heated at 95°C for 1 h. After cooling, 5.0 mL of butanol was added and the mixture was shaken vigorously and centrifuged at 3200 rpm for 15 min. The colour extracted with butanol layer was read at 535 nm. Standard solution (1–5 nmol) in a 4.0-mL volume and blank containing 4 mL distilled water were processed along with test samples.

Estimation of TBARS in heart

TBARS in myocardium was estimated by the method of Fraga et al (1988). To 0.5 mL tissue homogenate, 0.5 mL saline and 1 mL 10% trichloroacetic acid (TCA) were added, mixed well and centrifuged at 3000 rpm for 20 min. To 1 mL of the protein-free supernatant, 0.25 mL of TBA reagent was added, the contents were mixed well and boiled for 1 h at 95°C. The tubes were then cooled to room temperature under running water and absorption measured at 532 nm.

Estimation of lipid hydroperoxides

Plasma and heart lipid hydroperoxides were estimated by the method of Jiang et al (1992). Fox reagent (0.9 mL; 88 mg butylated hydroxy toluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium iron (II) sulfate were added to 90 mL methanol and 10 mL of 250 mM sulfuric acid) was mixed with 0.1 mL of the tissue homogenate/0.1 mL of plasma and incubated for 30 min at room temperature. The colour developed was read at 560 nm.

Assay of SOD

The activity of SOD in heart tissue was assayed by the method of Kakkar et al (1984). Heart tissue was extracted with 25 mol L^{-1} sucrose and an initial purification was performed using ammonium sulfate. The ammonium sulfate-fractionated superoxide dismutase preparation was dialysed overnight against 0.0025 mol L^{-1} Tris hydrochloride buffer (pH 7.4) before being used for enzyme assay. The assay mixture contained 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.025 mol L^{-1}), 0.1 mL 186 μmol L^{-1} phenazine methosulfate, 0.3 mL 300 μmol L^{-1} nitroblue tetrazolium, 0.2 mL 780 μmol L^{-1} NADH and approximately diluted enzyme preparation and water in a total volume of 3 mL. After incubation at 30°C for 90 s, the reaction was terminated by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 mL *n*-butanol. The colour intensity of the chromogen in the butanol layer was measured at 560 nm against *n*-butanol.

Assay of catalase

The catalase activity in heart was assayed by the method of Sinha (1972). To 1.0 mL phosphate buffer, 0.1 mL enzyme preparation and 0.4 mL hydrogen peroxide were added. The reaction was stopped at 30 s by the addition of 2.0 mL dichromate-acetic acid reagent. The tubes were kept in boiling water bath for 10 min, cooled and read at 620 nm. A system devoid of enzyme served as control. A series of standards in the range 20–100 μmol H_2O_2 were also processed as above along with a blank containing distilled water.

Assay of glutathione peroxidase (GPx)

GPx in heart was assayed by the method of Rotruck et al (1973). To 0.2 mL buffer, 0.2 mL EDTA, 0.1 mL sodium azide and 0.2 mL tissue homogenate (homogenized in 0.4 M phosphate buffer, pH 7.0) were added. To this mixture, 0.2 mL glutathione, followed by 0.1 mL hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 min along with a control tube containing all reagents but no enzyme. After 10 min, the reaction was arrested by the addition of 0.4 mL 10% TCA. The tubes were centrifuged and the supernatant was assayed for glutathione content by using Ellman's reagent (19.8 mg of 5,5′-dithio bisnitrobenzoic acid (DTNB) in 100 mL of 0.1% sodium citrate).

Estimation of reduced glutathione (GSH)

GSH was estimated by the method of Ellman (1959). Plasma (0.5 mL) or heart homogenate (0.5 mL) was pipetted out and precipitated with 2.0 mL of 5% TCA. One millilitre of the supernatant was taken after centrifugation. To this was added 0.5 mL of Ellman's reagent and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 mL of buffer.

Estimation of vitamin C

Vitamin C was estimated by the method of Omaye et al (1979). Plasma (0.5 mL) or heart homogenate (0.5 mL) was mixed thoroughly with 1.5 mL of 6% TCA and centrifuged for 20 min at 3700 rpm. To 0.5 mL of the supernatant, 0.5 mL of 2,4 dinitro phenyl hydrazine (DNPH) reagent was added and mixed well. The tubes were allowed to stand at room temperature for 3 h, removed and they were later placed in ice-cold water. To this, 2.5 mL of 85% sulfuric acid was added and allowed to stand for 30 min. A set of standards containing 10–50 μg of ascorbic acid were processed similarly along with a blank containing 0.5 mL of 4% TCA. The colour developed was read at 530 nm.

Estimation of protein

The protein content of tissue homogenates was determined by the method of Lowry et al (1951). Tissue homogenate (0.5 mL) was mixed with 0.5 mL of 10% TCA and centrifuged for 10 min. The precipitate obtained was dissolved in 1.0 mL of 0.1 N NaOH. From this a volume of 0.1 mL was taken for protein estimation. This 0.1-mL sample was mixed with 5.0 mL of alkaline copper reagent and allowed to stand at room temperature for 10 min. Folin's phenol reagent (0.5 mL) was added and the blue colour developed was read after 20 min at 640 nm.

Statstical analysis

Statistical analysis was performed using one-way analysis of variance by SPSS software 12.00, followed by Duncan's Multiple Range Test (DMRT). Results were expressed as mean ± s.d. from six rats in each group. $P < 0.05$ was considered to be significant.

Results

Table 1 shows the effect of rutin on the activity of creatine kinase, LDH, AST and ALT in the serum of normal and isoproterenol-treated rats. Isoproterenol-treated rats showed a significant ($P < 0.05$) increase in the activity of creatine kinase, LDH, AST and ALT in serum when compared with normal rats. Pretreatment with rutin (40 or 80 mg kg^{-1}) for 42 days significantly ($P < 0.05$) decreased the activity of these enzymes in the serum of isoproterenol-treated rats.

Table 2 shows the activity of creatine kinase, LDH, AST and ALT in the heart in normal and isoproterenol-treated rats. Isoproterenol induction caused a significant ($P < 0.05$) decrease in the activity of these enzymes in heart when compared with normal rats. Pretreatment with rutin (40 or 80 mg kg^{-1}) for 42 days significantly ($P < 0.05$) increased the activity of these enzymes in the heart of isoproterenol-treated rats.

Table 3 illustrates the effect of rutin on plasma and myocardial TBARS and lipid hydroperoxides in normal and isoproterenol-treated rats. Isoproterenol-treated rats showed a significant increase ($P < 0.05$) in TBARS and lipid hydroperoxides in plasma and heart when compared with normal rats. Pretreatment with rutin (40 or 80 mg kg^{-1}) to isoproterenol-treated rats for 42 days significantly ($P < 0.05$) decreased both the levels of TBARS and lipid hydroperoxides.

Table 4 represents the effect of rutin on myocardial antioxidants, such as SOD, catalase and GPx, in normal and isoproterenol-induced rats. Rats treated with isoproterenol showed a significant ($P < 0.05$) decrease in the activity of these enzymic antioxidants in the heart as compared with normal rats. Pretreatment with rutin (40 or 80 mg kg^{-1}) to isoproterenol-treated rats for 42 days exerted a significant ($P < 0.05$) increase in the activity of these enzymes.

Table 5 shows the effect of rutin on plasma and heart vitamin C and GSH in normal and isoproterenol-induced rats. Rats treated with isoproterenol, showed a significant decrease in the concentration of vitamin C and GSH in plasma and heart as compared with normal rats. Administration of rutin (40 or 80 mg kg^{-1}) to isoproterenol-treated rats for 42 days significantly ($P < 0.05$) increased the concentration of these antioxidants.

In our study, for all the parameters studied, the effect of rutin at a dose of 80 mg kg^{-1} was more pronounced than that at a dose of 40 mg kg^{-1} in isoproterenol-induced rats. Treatment with rutin at doses of 40 and 80 mg kg^{-1} to normal rats did not have any significant effect.

Discussion

Our study shows that rutin pretreatment of isoproterenol-induced rats prevents the rise in lipid peroxides and decrease in antioxidants. The isoproterenol-induced rats model of myocardial necrosis is widely used to evaluate cardioprotective drugs and to study myocardial consequences of ischaemic disorders (Cao et al 1994). Oxidative stress can damage many biological molecules and, indeed, proteins and DNA are more often significant targets of injury than are lipids, with lipid peroxidation often occurring late in the injury process (Halliwell & Chirico 1993). Damage to the myocardium could be due to the induction of free-radical-mediated lipid peroxidation by isoproterenol. Hence, therapeutic intervention with an antioxidant may be useful in preventing these deleterious changes as a consequences of isoproterenol administration (Mohanthy et al 2004).

Table 1 Effect of rutin on serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in normal and isoproterenol (ISO)-induced myocardial infarction in rats

Groups	CK (IU L^{-1})	LDH (IU L^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
Normal untreated	226.06 ± 19.98^{a}	79.51 ± 2.33^{a}	23.23 ± 2.17^{a}	11.45 ± 0.72^{a}
Normal + rutin 40 mg kg^{-1}	221.35 ± 19.30^{a}	78.57 ± 2.23^{a}	23.65 ± 2.33^{a}	11.21 ± 0.72^{a}
Normal + rutin 80 mg kg^{-1}	223.71 ± 10.65^{a}	79.39 ± 1.72^{a}	22.10 ± 1.91^{a}	11.28 ± 0.98^{a}
ISO alone	355.61 ± 20.79^{b}	146.43 ± 8.33^{b}	43.19 ± 2.63^{b}	27.38 ± 1.85^{b}
Rutin 40 mg kg^{-1} + ISO	320.23 ± 26.24^{c}	129.37 ± 7.00^{bc}	35.96 ± 2.77^{c}	20.35 ± 1.30^{c}
Rutin 80 mg kg^{-1} + ISO	279.71 ± 15.32^{d}	93.61 ± 2.06^{d}	30.91 ± 2.60^{d}	18.23 ± 1.01^{d}

Rutin was administered to rats for a period of 42 days. Values are mean ± s.d. for 6 samples. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Table 2 Effect of rutin on heart creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in normal and isoproterenol (ISO)-induced myocardial infarction in rats

Groups	CK (μmol phosphorous liberated/min/mg protein)	LDH (nmol pyruvate liberated/min/mg protein)	AST (nmol pyruvate liberated/min/mg protein)	ALT (nmol pyruvate liberated/min/mg protein)
Normal untreated	16.41 ± 0.92^{a}	109.60 ± 5.08^{a}	33.78 ± 2.69^{a}	22.54 ± 2.04^{a}
Normal + rutin 40 mg kg^{-1}	16.38 ± 0.57^{a}	107.71 ± 3.33^{a}	32.91 ± 2.78^{a}	21.25 ± 2.07^{a}
Normal + rutin 80 mg kg^{-1}	16.99 ± 0.61^{a}	109.92 ± 3.08^{a}	33.39 ± 2.69^{a}	22.22 ± 2.13^{a}
ISO alone	7.46 ± 0.66^{b}	77.90 ± 4.98^{b}	22.55 ± 2.05^{b}	11.08 ± 1.21^{b}
Rutin 40 mg kg^{-1} + ISO	11.67 ± 0.35^{c}	90.41 ± 5.07^{c}	28.66 ± 2.54^{c}	16.25 ± 1.42^{c}
Rutin 80 mg kg^{-1} + ISO	13.84 ± 0.55^{cd}	102.05 ± 3.99^{a}	31.02 ± 2.54^{ad}	19.15 ± 0.98^{d}

Rutin was administered to rats for a period of 42 days. Values are mean ± s.d. for 6 samples. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Table 3 Effect of rutin on plasma and heart thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HP) in normal and isoproterenol (ISO)-induced myocardial infarction in rats

Groups	TBARS (nmol mL^{-1})	HP (× 10^{-5} mmol dL^{-1})	TBARS (mM/100 g wet tissue)	HP (mM/100 g wet tissue)
Normal untreated	1.72 ± 0.11^{a}	7.95 ± 0.56^{a}	0.60 ± 0.04^{a}	16.73 ± 0.75^{a}
Normal + rutin 40 mg kg^{-1}	1.71 ± 0.84^{a}	7.85 ± 0.86^{a}	0.59 ± 0.03^{a}	16.11 ± 0.52^{a}
Normal + rutin 80 mg kg^{-1}	1.64 ± 0.45^{a}	7.91 ± 0.34^{a}	0.58 ± 0.04^{a}	16.50 ± 0.57^{a}
ISO alone	4.96 ± 0.34^{b}	15.50 ± 0.24^{b}	1.02 ± 0.06^{b}	26.90 ± 1.25^{b}
Rutin 40 mg kg^{-1} + ISO	3.38 ± 0.26^{c}	12.25 ± 0.91^{c}	0.88 ± 0.04^{c}	21.05 ± 3.29^{c}
Rutin 80 mg kg^{-1} + ISO	2.39 ± 0.32^{d}	9.35 ± 0.90^{a}	0.71 ± 0.04^{d}	19.63 ± 1.73^{d}

Rutin was administered to rats for a period of 42 days. Values are mean ± s.d. for 6 samples. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Table 4 Effect of rutin on superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in heart in normal and isoproterenol (ISO)-induced myocardial infarction in rats

Groups	SOD (U (mg protein)$^{-1}$)	Catalase (μmol of H_2O_2 consumed/ min/mg protein)	GPx (μg of GSH consumed/ min/mg protein)
Normal untreated	12.11 ± 0.51^{a}	7.61 ± 0.50^{a}	1.85 ± 0.53^{a}
Normal + rutin 40 mg kg^{-1}	11.80 ± 0.93^{a}	7.87 ± 0.43^{a}	1.82 ± 0.18^{a}
Normal + rutin 80 mg kg^{-1}	11.14 ± 0.89^{a}	7.54 ± 0.44^{a}	1.79 ± 0.11^{a}
ISO alone	5.07 ± 0.49^{b}	3.91 ± 0.26^{b}	0.82 ± 0.39^{b}
Rutin 40 mg kg^{-1} + ISO	7.24 ± 0.43^{c}	5.11 ± 0.34^{c}	1.21 ± 0.61^{c}
Rutin 80 mg kg^{-1} + ISO	9.25 ± 0.43^{d}	6.28 ± 0.28^{d}	1.50 ± 0.02^{d}

SOD units: one unit is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in 1 min. Rutin was administered to rats for a period of 42 days. Values are mean ± s.d. for 6 samples. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Effect on cardiac marker enzymes

The cardiac marker enzymes of MI are creatine kinase, LDH, AST and ALT. Rats administered with isoproterenol showed an increase in the activity of these enzymes in serum and a decrease in the activity of these enzymes in heart. The increase in the activity of marker enzymes in serum could be due to leakage of these enzymes from the heart as a result of isoproterenol-induced necrosis. Pretreatement with rutin had a significant effect on these enzymes in serum and heart of isoproterenol-induced rats.

Effect on lipid peroxidation

Lipid peroxides play an important role in myocardial cell damage. Enormous amounts of reactive oxygen species (ROS), like superoxide, hydrogen peroxide and hydroxyl radicals, are produced during MI. Significant elevation in the concentration of TBARS and lipid hydroperoxides was observed in isoproterenol-treated rats. Lipid peroxidation is an important pathogenic event in MI and accumulation of lipid peroxides reflects the various stages of this disease and its complications (Hamberg et al 1974). It

Table 5 Effect of rutin on plasma and heart reduced glutathione (GSH) and vitamin C in normal and isoproterenol (ISO)-induced myocardial infarction in rats

Groups	GSH (mg dL^{-1})	GSH (mmol (g wet tissue)$^{-1}$)	Vitamin C (mg dL^{-1})	Vitamin C mg (g wet tissue)$^{-1}$)
Normal untreated	28.27 ± 2.32^{a}	8.64 ± 0.16^{a}	229.69 ± 15.39^{a}	1.76 ± 0.29^{a}
Normal + rutin 40 mg kg^{-1}	28.37 ± 2.86^{a}	8.70 ± 0.58^{a}	235.88 ± 16.19^{a}	1.80 ± 0.48^{a}
Normal + rutin 80 mg kg^{-1}	28.87 ± 1.95^{a}	8.92 ± 0.60^{a}	238.33 ± 16.33^{a}	1.88 ± 0.37^{a}
ISO alone	16.15 ± 1.54^{b}	4.09 ± 0.47^{b}	121.04 ± 8.46^{b}	0.86 ± 0.32^{b}
Rutin 40 mg kg^{-1} + ISO	21.28 ± 2.65^{c}	5.86 ± 0.28^{c}	172.92 ± 10.12^{c}	1.13 ± 0.28^{c}
Rutin 80 mg kg^{-1} + ISO	25.04 ± 2.51^{d}	7.19 ± 0.58^{d}	205.40 ± 9.98^{d}	1.40 ± 0.51^{d}

Rutin was administered to rats for a period of 42 days. Values are mean ± s.d. for 6 samples. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

is well known that isoproterenol produces free radicals and that these free radicals are involved in membrane damage, leading to increased levels of TBARS and lipid hydroperoxides. On treatment with rutin, the levels of TBARS and lipid hydroperoxides were decreased in isoproterenol-treated rats. Rutin inhibits lipid peroxide formation and free radical production. The free radical inhibitory activity of rutin is attributed to its antioxidant property (Liao & Yin 2000), which effectively scavenges the ROS and decreases lipid peroxidation end products.

Effect on enzymic antioxidants

In-vivo and in-vitro studies, as well epidemiological studies, suggest an inverse correlation between the severity of oxidative-stress-induced diseases and levels of antioxidants (Oka et al 1999). Both enzymic and non-enzymic antioxidants are altered in isoproterenol-induced MI.

Free radical scavenging enzymes such as SOD, catalase and GPx are the first line of cellular defence against oxidative injury. The equilibrium between these enzymes is an important factor for the effective removal of ROS in intracellular organelles (Andrew & Mathew 1989). Isoproterenol-treated rats showed decreased activity of SOD and catalase in heart. A decrease in the activity of these antioxidant enzymes can lead to the formation of oxygen and hydrogen peroxide, which in turn can form the toxic hydroxy radical ($OH^{\cdot}$). The decrease in the activity of SOD and catalase may be due to myocardial cell damage. The increased activity of the myocardial catalase and SOD is associated with decreased levels of lipid peroxidation in the rutin (40 or 80 mg kg^{-1})-treated isoproterenol group. This can result in decreased formation of toxic intermediates.

The enzyme glutathione peroxidase is a well-known first line of defence against oxidative stress, which in turn requires glutathione as co-factor. GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2. The observed decreased activity of GPx in the study might be due to increased concentration of hydroperoxides or due to decreased concentration of GSH in isoproterenol-induced rats. Oral pretreatment with rutin increases the activity of GPx in isoproterenol-treated rats.

Effect on non-enzymic antioxidants

The second line of defence consists of the non-enzymatic scavengers, viz. ascorbic acid and reduced glutathione containing compounds, which scavenge residual free radicals escaping decomposition by the antioxidant enzymes (Kloner et al 1974).

Reduced glutathione (GSH) is one of the major constituents of erythrocytes and plays an important role in providing protection against oxidative damage. The antioxidant function of the tripeptide is related to oxidation of the thiol group of its cysteine residue with formation of a disulfide (GSSG). It has been proposed that antioxidants, which maintain the concentration of GSH, may restore the cellular defence mechanism, block lipid peroxidation and protect the tissues against oxidative damage (Chugh et al 1999). A decrease in the concentration of GSH in isoproterenol-treated rats might be due to its utilization by the glutathione-dependent antioxidant process.

Vitamin C (ascorbic acid) is an important water-soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body (Jaffe 1984). Plasma devoid of vitamin C, but no other endogenous antioxidant, is extremely vulnerable to oxidant stress and susceptible to peroxidative damage to lipids (Frei et al 1989). Vitamin C neutralizes reactive oxygen metabolites and reduces oxidative DNA damage (Frei 1994). The decreased concentration of vitamin C in isoproterenol-treated rats might be due to increased lipid peroxides.

During isoproterenol-induced myocardial necrosis, the levels of enzymatic and non-enzymatic antioxidants decrease significantly leading to increased free radical formation. These radicals cascade a number of reactions that could be harmful to the myocardium (Samuelson 1977). Earlier studies have shown that isoproterenol administration produces free radicals (Singal et al 1983) and, via β-adrenoreceptor mechanism, affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial cell necrosis. These cytotoxic free radicals exert unfavourable influences on the heart. The increase in the activity of enzymatic antioxidants in isoproterenol-induced rats treated with rutin (40 or

80 mg kg^{-1}) could prevent free radical formation during myocardial necrosis. These results show the antioxidant activity of rutin.

Conclusion

Our results show that rutin prevented the increase in lipid peroxides and prevented the decrease in both enzymatic and non-enzymatic antioxidants in isoproterenol-induced rats. This effect might be due to the antioxidant activity of rutin.

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