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Preventive effects of rutin on lysosomal enzymes in isoproterenol induced cardio toxic rats: Biochemical, histological and in vitro evidences

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ABSTRACT

This study was aimed to evaluate the preventive effect of rutin on lysosomal enzymes in isoproterenol induced cardio toxic rats. Male albino Wistar rats were pretreated with rutin (80 mg/kg) daily for a period of 42 days. After the pretreatment period, isoproterenol (100 mg/kg) was subcutaneously injected to rats at an interval of 24 h for two days. The activity of serum creatine kinase-MB and the levels of serum troponins T and I and the activities of serum and heart lysosomal enzymes (β-glucuronidase, β-N-acetylglucosaminidase, β-galactosidase, cathepsin-B and D) were increased significantly ($P<0.05$) in isoproterenol induced cardio toxic rats. Isoproterenol induced cardio toxic rats also resulted in decreased stability of membranes, which was reflected by decreased activities of β-glucuronidase and cathepsin-D in mitochondrial, nuclear, lysosomal and microsomal fractions. Pretreatment with rutin daily for a period of 42 days to isoproterenol induced cardio toxic rats prevented the changes in the activities of these enzymes. Oral treatment with rutin (80 mg/kg) to normal rats did not show any significant effect in all the biochemical parameters studied. Histopathology of myocardium showed the preventive effects of rutin in isoproterenol induced cardio toxic rats. *In vitro* study also confirmed the mechanism of action of rutin. Thus, the results of our study show that rutin protects the lysosomal membrane against isoproterenol induced cardiac damage. The observed effects are due to the free radical scavenging, antioxidant and membrane stabilizing properties of rutin.

1. Introduction

Myocardial infarction affects a large population in the world. Myocardial ischemia occurs when myocardial oxygen demand exceeds oxygen supply and as a result it causes cell injury known as myocardial infarction (Mohanty et al., 2004). Isoproterenol causes severe stress in the myocardium, results in infarct like necrosis of the heart muscle (Sushamakumari et al., 1989). It is well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membranes (Chein et al., 1978).

Dietary factors play an important role in the development of various human diseases, including cardiovascular diseases. A scientific study has shown that diets rich in fruits, herbs and spices are associated with a reduced risk of cardiovascular diseases (Banerjee and Maulik, 2002). Rutin (Fig. 1), a bioflavonoid, 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, anti-inflammatory, anti-diarrhoeal, anti-ulcer,anti-mutagenic, vasodilator, immunomodulatory and hepatoprotective (Janbaz et al., 2002). In our laboratory, we previously reported the beneficial effects of rutin in streptozotocin induced diabetic rats (Kamalakkannan and Stanely Mainzen Prince, 2006a, b, c; Stanely Mainzen Prince and Kamalakkannan, 2006; Stanely Mainzen Prince and Kannan, 2006). We also reported the preventive effects of rutin on lipids, lipoproteins, adenosine triphosphatases, lipid peroxides and antioxidants in isoproterenol induced rats (Karthick and Stanely Mainzen Prince, 2006; Stanely Mainzen Prince and Karthick, 2007).

Considerable attention has been focused on lysosomal alterations that might accompany ischemic or hypoxic myocellular damage. Lysosomal enzymes play an important role in the inflammatory process. Isoproterenol induced myocardial infarction results in increased lysosomal hydrolases activity that may be responsible for tissue damage and infarcted heart (Ravichandran et al., 1990). There is a report showing that isoproterenol induced myocardial necrosis involves membrane permeability alterations that bring about loss of function and integrity of myocardial membranes (Todd et al., 1980). Rutin is known to have membrane stabilization action (Stanely Mainzen Prince and Karthick, 2007), and it is possible that stabilization of myocardial cell membranes, particularly the lysosomal membranes, may prolong the viability of ischemic cardiac muscle. Literature survey has shown that there is no scientific report available on the effects of rutin on lysosomal enzymes in myocardial infarction. In continuation of our research work on rutin, the present investigation was undertaken to study the preventive effects of rutin in reducing the extent of lysosomal damage in the myocardium of isoproterenol induced cardio toxic rats. In addition to this, we carried

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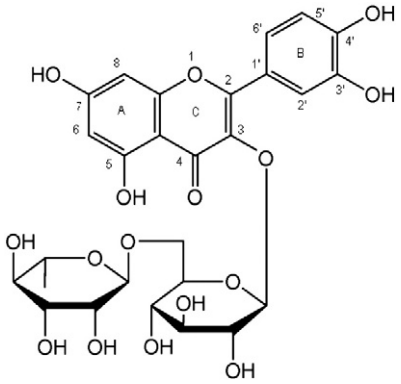

Fig. 1. Structure of rutin (5, 7, 3′, 4′-OH, 3-rutinose).

out the preventive effects of rutin on serum cardiac diagnostic markers and histopathology of myocardium. *In vitro* study on the effects of rutin on scavenging 1, 1-diphenyl-2-picryl hydrazyl radical and 2, 2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical were determined to know the deeper mechanism of action of rutin.

2. Materials and methods

2.1. Drugs and chemicals

Rutin hydrate, isoproterenol hydrochloride, p-nitrophenyl-*N*-acetyl-β-D-glucosaminide, sodium dodecyl sulphate, p-nitrophenyl-β-D-glucuronide, 1, 1-diphenyl-2-picryl hydrazyl radical and 2, 2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical were purchased from Sigma Chemical Co., St. Louis, MO, USA. All the other chemicals used were of high analytical grade.

2.2. Experimental animals

All the experiments were carried out with male albino Wistar rats (150–170 g) obtained from Central Animal House, Rajah Muthiah Institute of Health Sciences, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47×34×20 cm) lined with husk, renewed every 24 h, under a light-dark cycle at around 22 °C and had free access to tap water and food. The rats were fed on a standard pelleted diet (Pranav Agro Industries Ltd, Maharashtra, India). The diet provided a metabolizable energy of 3000 kcal/kg. 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 Animal Ethical Committee of Annamalai University.

2.3. Preparation of cardio toxic rats

Isoproterenol (100 mg/kg) was dissolved in saline and subcutaneously injected to rats at an interval of 24 h for two days. Isoproterenol induced cardio toxicity was confirmed by elevated levels of serum creatine kinase and lactate dehydrogenase in rats.

2.4. Experimental design

The rats were divided into four groups of eight rats as follows. Two rats from each group were used for histopathological study. Group I: normal untreated rats, Group II: rats were treated with rutin (80 mg/kg) daily for a period of 42 days. Group III: rats were subcutaneously injected with isoproterenol (100 mg/kg) dissolved in saline at an interval of 24 h for two days (on 43rd and 44th day). Group IV: rats were pretreated with rutin (80 mg/kg) daily for a period of 42 days and then injected with isoproterenol (100 mg/kg) at an interval of 24 h for two days (43rd and 44th day). Rutin was suspended in carboxy methyl cellulose 0.01 g/l and was orally administered to rats using an intragastric tube (1 ml/rat) daily for a period of 42 days. Normal control and isoproterenol control rats were received carboxy methyl cellulose alone (1 ml/rat) daily for a period of 42 days.

At the end of the experimental period, after twelve hours of second dose of isoproterenol-injection (on 45th day), all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected and serum was separated for various biochemical estimations. The heart tissue was dissected out immediately and stored for the separation of subcellular fractions. The duration and dose of rutin pretreatment was based on our earlier study (Stanely Mainzen Prince and Karthick, 2007).

2.5. Separation of subcellular fractions

The heart tissue samples were cut open and placed in isotonic saline to remove the blood. Then, the heart tissues were rinsed in ice cold 0.25 M sucrose at 4 °C. A portion of this preparation was used to determine the total activity. Another portion of the homogenate was subjected to differential centrifugation and the different fractions were separated as follows: structural proteins, nucleus, and cell debris at 600×*g* for 10 min; mitochondria at 5000×*g* for 10 min; lysosomes at 15,000×*g* for 10 min; microsomes at 120,000×*g* for 30 min and supernatant, the cytosol. Myocardial subfractions were treated with Triton X-100 (final concentration 0.2% v/v) in ice for 15 min prior to the determination of enzymic activities (Sathish et al. 2003; Devika and Stanely Mainzen Prince, 2008; Senthil Kumaran and Stanely Mainzen Prince, 2010).

2.6. Assay of cardiac marker enzyme

Activity of serum creatine kinase-MB was measured by a standard commercial kit.

2.7. Estimation of serum cardiac troponins

The levels of serum cardiac troponins T and I were estimated by chemiluminescence immunoassay using standard kits (Roche Diagnostics, Switzerland).

2.8. Assay of lysosomal enzymes in the serum, heart and subcellular fractions and estimation of protein in the heart and subcellular fractions

The activity of β-glucuronidase in the serum, heart tissue homogenate and subcellular fractions were assayed according to the method of Kawai and Anno (1971). The activities of β-N-acetyl glucosaminidase, β-galactosidase and cathepsin-B in the serum and heart tissue homogenate were assayed by the method of Moore and Morris (1982), Conchie et al. (1967) and Barrett (1972). Cathepsin-D activity in the serum, heart tissue homogenate and subcellular fractions were assayed by the method of Sapolsky et al. (1973). The content of the protein in the heart tissue homogenate and subcellular fractions was determined by the method of Lowry et al. (1951).

2.9. Histopathological examination

The heart tissues obtained from all experiment groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the heart tissues were processed embedding in paraffin. Then, the heart tissues were sectioned and stained with haematoxylin and eosin and examined under high power microscope (×200) and photomicrographs were taken.