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Diosmin exhibits anti-hyperlipidemic effects in isoproterenol induced myocardial infarcted rats

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ABSTRACT

The aim of the present study was to evaluate the protective effects of diosmin on experimentally induced myocardial infarcted rats. Diosmin (5 and 10 mg/kg body weight) was administered orally as pretreatment daily for a period of 10 days. Then isoproterenol (100 mg/kg) was injected subcutaneously into rats at an interval of 24 h for 2 days (on 11th and 12th day). Isoproterenol-induced myocardial infarcted rats showed significant changes in electrocardiogram and an increase in the levels of cardiac markers, compared with normal rats. Additionally, increased plasma lipid peroxidation products and altered lipid metabolism in the plasma were observed in the isoproterenol-induced myocardial infarcted rats. Pretreatment with diosmin (5 and 10 mg/kg body weight) minimized the electrocardiographic changes, decreased the levels of serum cardiac marker enzymes reduced plasma lipid peroxidation and minimized the alterations in the lipid metabolism of isoproterenol-induced myocardial infarcted rats. Also, diosmin inhibited the enhanced activity of liver HMG CoA reductase. The *in vitro* study revealed the free radical scavenging activity of diosmin. The free radical scavenging and anti-hyperlipidaemic effects are the reasons for the cardioprotective effects of diosmin.

1. Introduction

Myocardial infarction is a disease that occurs when the coronary arteries reduce the blood supply to a part of the myocardium that leads to hypoxia (Sangeetha and Darlin Quine, 2008). Cardiovascular risk factors have long been studied in order to gain insight into the pathophysiology, epidemiology and therapy of acute myocardial infarction (López Messa et al., 2004). As data from the original Framingham cohort were analyzed, newer cohorts were added over the years, such as life style and diet, smoking, high blood pressure, altered lipid profile and obesity as important risk factors for myocardial infarction (O'Donnell and Elosua, 2008).

Therapeutic doses of isoproterenol (L-β-(3, 4-dihydroxyphenyl)-α-isopropyl aminoethanol hydrochloride), a β-adrenergic receptor agonist are regulating heart function. But in excessive dose, isoproterenol depletes the energy reserve of cardiomyocytes and thus results in biochemical and structural changes, which are responsible for the development of irreversible damage (Aman Upaganlawar et al., 2011). Isoproterenol also increases the intracellular levels of calcium, thereby causing myocardial infarction. It serves as a well accepted and standardized model to study the cardio protective effects of new drugs in preclinical trials.

The existing pharmacotherapy is effective in managing the myocardial infarction but with multiple adverse effects and drug–drug interactions (Wiffen et al., 2002). These disadvantages have enhanced the need for alternative phytoconstituents, which may be used to prevent the cardiovascular diseases. Flavonoids are present in fruits and various plants, which are pharmacologically active and can be used for the treatment of degenerative diseases. They have multiple biological activities including vasodilator (Duarte et al., 1993), anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, antiallergic, and antiviral effects (Brown, 1980; Middleton and Kandaswami, 1992). In general, antioxidant activity of flavonoids is closely related to preventive effects on various diseases including arteriosclerosis, liver injury, and carcinogenesis (Ho et al., 1994).

Diosmin (3′, 5,7-Trihydroxy-4′-methoxyflavone 7-rutinoside) is an unsaturated flavone that can be found mainly in Hyssop and Rosemary (Del Baño et al., 2004; Camarda et al., 2007). It exhibits anti- inflammatory, antioxidant and anti-mutagenic properties (Kuntz et al., 1999) Literature survey revealed that there are no scientific reports available on the effects of diosmin in myocardial infarction. The aim of the study is to understand the protective effect of diosmin in isoproterenol induced myocardial infarcted rats.

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2. Materials and methods

2.1. Experimental animals

All the experiments were carried out with healthy male albino Wistar rats (*Rattus norvegicus*) weighing 180–200 g, purchased from Mahaveer Enterprises, Hyderabad, India. They were housed in polypropylene cages ($47 \times 34 \times 20$ cm^3) (3 rats per cage) 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 and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Pune, and Maharashtra, India). The experiments were 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 (Approval no. 02; 10/06/2011).

2.2. Chemicals

Diosmin, a rhamnoglucoside (rutinoside), extracted from citrus species at 95% purity, and isoproterenol were purchased from Sigma Chemical Co., St. Louis, MO, USA. Sodium sulfite, dimethyl sulfoxide, potassium tetra borate, nitroblue tetrazolium and hydroxylamine hydrochloride were purchased from Himedia, Mumbai, India. All other chemicals and reagents used in the study were of analytical grade.

2.3. Induction of experimental myocardial infarction

Isoproterenol (100 mg/kg body weight) was dissolved in saline and injected subcutaneously into rats at an interval of 24 h for 2 days to induce myocardial infarction (Punithavathi and Stanely Mainzen Prince, 2010; Stanely Mainzen Prince, 2011).

2.4. Experimental design

The rats were divided into six groups of six rats each. Group I: normal rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group II: rats were treated with diosmin (5 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group III: rats were treated with diosmin (10 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days; Group IV: rats were given 2 ml of saline orally by gastric intubation daily for a period of 10 days and then injected subcutaneously with isoproterenol (100 mg/kg) in 2 ml of saline at an interval of 24 h for 2 days (on 11th and 12th day); Group V: rats were pretreated with diosmin (5 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then injected subcutaneously with isoproterenol (100 mg/kg) at an interval of 24 h for 2 days (on 11th and 12th day); Group VI: rats were pretreated with diosmin (10 mg/kg) dissolved in 2 ml of saline orally by gastric intubation daily for a period of 10 days and then injected subcutaneously with isoproterenol (100 mg/kg) at an interval of 24 h for 2 days (on 11th and 12th day).

2.5. Electrocardiogram

Electrocardiographic patterns in the normal and experimental rats were recorded according to the method of Mari Kannan and Darlin Quine (2011). Twenty four hours after the second dose of isoproterenol, rats of all the groups were anesthetized with ketamine hydrochloride (100 mg/kg body weight) intraperitoneally and the electrocardiographic patterns were recorded by a 16 channel polygraph (Biopac systems Inc., USA). The types of alterations (P wave, QRS complex, ST-segment elevation, RR interval) in the normal and experimental rats were recorded.

2.6. Analysis of cardiac markers.

The level of cardiac troponin-I in the serum was estimated by VITROS immunodiagnostic kit purchased from the Ortho-Clinical Diagnostics, Inc. New York, USA. Creatine kinase-MB in the serum was estimated by a standard diagnostic kit obtained from Accurex Private Ltd, Mumbai, India.

2.7. Estimation of lipid peroxidation products

The levels of plasma thiobarbituric acid reactive substances were estimated by the method of Yagi et al. (1998). Four ml of 0.083 N sulfuric acid were added to 0.5 ml of plasma. To this mixture, 0.5 ml of 10% phosphotungstic acid was added and mixed thoroughly. After allowing the tubes to stand at room temperature for 5 min, the mixture was centrifuged at $3000 \times g$ for 10 min. The supernatant was discarded and the sediment was mixed with 2 ml of sulfuric acid and 0.3 ml of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at $3000 \times g$ for 10 min. The sediment was suspended in 4 ml of double distilled water and 1 ml of thiobarbituric acid reagent was added. The reaction mixture was heated at 95 °C for 60 min. After cooling, 5 ml of n-butanol was added and the mixture was shaken vigorously and centrifuged at $3000 \times g$ for 15 min. The color extracted in the n-butanol layer was measured at 530 nm in a UV-Spectrophotometer.

Lipid hydroperoxides in the plasma were estimated by the method of Jiang et al. (1992). 1.8 ml of Fox reagent was added to 0.2 ml of plasma and incubated for 30 min at room temperature and the absorbance was measured at 560 nm in a UV-Spectrophotometer.

2.8. Estimation of lipid profile

Lipid profile was estimated in the plasma and heart tissue homogenates. Lipids were extracted from the heart tissue by the method of Folch et al. (1957). This lipid extract was used for further analysis. The total plasma cholesterol and triglyceride contents were estimated by reagent kits (Qualigens Diagnostics, Mumbai, India). Plasma high density lipoprotein (HDL) level was estimated by a reagent kit (Auto Zyme: HDL-Cholesterol) from Accurex Diagnostics Private Limited, Mumbai, India. Plasma low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were calculated as follows: LDL–cholesterol=Total cholesterol– (HDL-cholesterol +VLDL-cholesterol); VLDL-cholesterol=Triglycerides/5 (Rajadurai and Stanely Mainzen Prince, 2006).

2.9. Assay of 3-hydroxy-3 methyl glutaryl CoA reductase (HMG CoA reductase)

The activity of HMG-CoA reductase was assayed in the liver by the method of Rao and Ramakrishnan (1975). The ratio of HMG CoA to mevalonate was taken as an index of enzyme activity which catalyzes the conversion of β-hydroxy-β-methyl glutaryl CoA to mevalonate. Lower ratio of β-hydroxy-β-methyl glutaryl CoA to mevalonate indicates higher enzyme activity and higher ratio of β-hydroxy-β-methyl glutaryl CoA to mevalonate indicates lower enzyme activity.

2.10. Assessment of cardiac indices and left ventricular hypertrophy

The heart was then removed, cleaned from fat and fibrous tissue, and dried with filter paper before determining the weight of the whole heart and the left ventricle. Cardiac indices were