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Crocus sativus L. (saffron) attenuates isoproterenol-induced myocardial injury via preserving cardiac functions and strengthening antioxidant defense system

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ABSTRACT

Saffron (dried stigmas of *Crocus sativus* L.), a naturally derived plant product, has long been used as a traditional ancient medicine against various human diseases. The aim of the series of experiments was to systematically determine whether saffron exerts cardioprotection in isoproterenol-induced myocardial damage. Male Wistar rats (150–175 g) were divided into five groups: control, isoproterenol (ISO) and three saffron (200, 400 and 800 mg/kg) treatment groups. Aqueous extract of saffron or vehicle was administered orally to rats for four weeks. On days 28 and 29, the animals in ISO and saffron treatment groups were administered ISO (85 mg/kg, s.c.) at an interval of 24 h. On day 30, after recording hemodynamics and left ventricular functions, animals were sacrificed for biochemical, histopathological and electromicroscopical examinations. Isoproterenol challenged animals showed depressed hemodynamics and left ventricular functions as evident by decreased left ventricular rate of peak positive and negative pressure change and elevated left ventricular end-diastolic pressure. Structural and ultrastructural studies further confirmed the damage which was reconfirmed by increased thiobarbituric acid reactive substances ($p < 0.001$) and decreased creatine kinase-MB and lactate dehydrogenase ($p < 0.001$). In addition, significant reduction in superoxide dismutase and catalase ($p < 0.001$) was observed in ISO group. Our results suggested that saffron at all the doses exerted significant cardioprotective effect by preserving hemodynamics and left ventricular functions, maintaining structural integrity and augmenting antioxidant status. Among the different doses used, saffron at 400 mg/kg dose exhibited maximum protective effects which could be due to maintenance of the redox status of the cell reinforcing its role as an antioxidant.

1. Introduction

Isoproterenol, a synthetic catecholamine at higher doses produces diffuse myocardial necrosis, myofibrillar degeneration and leukocytic infiltration (Ferrans et al., 1964; Kahn et al., 1969). Prolonged catecholamine exposure causes myocardial hypertrophy in animals, even in sub-hypertensive doses (Alderman and Harrison, 1971). The histopathological lesions produced by isoproterenol resemble myocardial infarcts seen after acute myocardial infarction in humans (Milei et al., 1978). Other studies have demonstrated that the application of isoproterenol deteriorates hemodynamic variables such as left ventricular end-diastolic volume and pressure resulting in progressive left ventricular wall thickness (Teerlink et al., 1994). There are various proposed mechanisms of isoproterenol-induced myocardial injury including mismatch of oxygen supply versus demand following coronary hypotension and myocardial hyperactivity (Yeager and Iams, 1981) and excessive production of free radicals from oxidative metabolism of catecholamines (Remião et al., 2001). These free radicals have been culpably involved in oxidative ischemic injury and are the central component of cellular damage that severely affects the myocardium. As a consequence, a great deal of research is focused on the prevention of diseases in which free radicals contribute significantly in pathophysiology (atherosclerosis, ischemia–reperfusion injury, hypertension, diabetic cardiomyopathy, hypertrophy and congestive heart failure) and their modulation by antioxidants (Bahorun et al., 2006).

At present natural medicine and herbal drugs are acquiring much attention as potential source of antioxidants. They serve as excellent candidates against reactive oxygen species (ROS)-

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induced pathologies. *Crocus sativus* L., commonly known as saffron is one such popular medicinal plant. It is widely used to promote health and fight diseases especially in the Middle East and Southeast Asia. Saffron extract constitutes of many carotenoids such as crocetin, crocetin di-glucose ester, crocetin gentiobiose glucose ester and crocin. It has been shown that saffron possesses antioxidant (Joukar et al., 2010), anticarcinogenic (Aung et al., 2007; Dhar et al., 2009), anti-inflammatory (Hosseinzadeh and Younesi, 2002) and immuno-modulating properties (Nair et al., 1995). The active ingredients behind these effects have been identified as crocin and crocetin.

Despite large number of studies, the exact mechanism underlying its therapeutic effects still lacks substantial data. In particular, no study has yet addressed its role in myocardial ischemic injury. The aim of the present study was to evaluate the cardioprotective potential of saffron in experimental model of myocardial ischemic injury. In the present study, isoproterenol was used to produce myocardial ischemia in experimental rats and saffron was administered before and during the onset of ischemia. To assess the deleterious effects of ischemic injury, various hemodynamic variables (SAP, MAP, DAP and HR) and ventricular functional parameters [(+) LVdP/dt, (−) LVdP/dt and LVEDP] were incorporated in the study. To further confirm the ischemic damage structural and ultrastructural studies were also carried out. This was followed by the biochemical evaluation of myocardial injury markers i.e. TBARS, CK-MB and LDH. To delineate the mechanism of protection, effect of saffron on endogenous antioxidant system was also studied.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 150–175 g, were used in the study. Animals were obtained from the Central Animal House Facility of All India Institute of Medical Sciences, New Delhi, India. They were kept in the departmental animal house under controlled conditions of temperature at 25 ± 2 °C, relative humidity of 60 ± 5% and light:dark cycle of 12 h each. The animals were fed chow pellets (Gulmohar Feed, Delhi, India) and allowed water *ad libitum*. Animals were maintained in polypropylene cages, each cage containing a maximum of four animals. The study was conducted in accordance with the protocol approved by the Institutional Animal Ethics Committee [375/IAEC/07].

2.2. Chemicals

Isoproterenol hemisulphate was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals were obtained from Sisco Research Laboratories (Mumbai, India). Isoproterenol hemisulphate solution (20%) was prepared under sterile condition with 0.9% saline immediately before injection and used within 30 min of preparation.

2.3. Preparation of saffron extract

Saffron was purchased from the Khari Baoli, local market of Delhi, India. The stigmas were identified by Dr. Santosh Kumari, Botantist, Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi. A voucher specimen was deposited in the Cardiovascular Laboratory, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi. Saffron stigmas (200 g) were powdered and extracted with water in soxhlet apparatus for 4 h. The extract was evaporated to dryness under reduced pressure to give solid residue. The residue was stored at room temperature for subsequent experiments.

2.4. Treatment schedule

The animals were randomly assigned to five experimental groups, with ten animals in each: control, isoproterenol and saffron (200, 400 and 800 mg/kg) treatment groups. Saffron was administrated to the rats in doses of 200, 400 and 800 mg/kg orally for 30 days, while animals in control and isoproterenol group received distil water for the same duration. On days 28 and 29, animals in isoproterenol and saffron treatment groups were administrated isoproterenol (85 mg/kg, s.c.) twice at an interval of 24 h while the animals in control group received saline injections subcutaneously by the same schedule. On day 30, hemodynamic variables were recorded. Thereafter, animals were sacrificed with an overdose of anesthesia; their hearts were removed and immediately processed for histopathological and ultrastructural studies. For performing biochemical estimations, six hearts were quickly frozen in liquid nitrogen and stored at −80 °C till further analysis.

2.5. Assessment of hemodynamic and left ventricular functions

The animals were anesthetized with pentobarbitone sodium (60 mg/kg, i.p.) and atropine (0.6 mg/kg, i.p.) was administered to maintain heart rate during the surgical procedure and to reduce tracheobronchial secretions. Body temperature was monitored and maintained at 37 °C throughout the experimental period. The neck was opened with a ventral midline incision and tracheostomy was performed. The rats were ventilated with room air from a positive pressure ventilator (Inco, India) at the rate of 90 strokes/min and a tidal volume of 10 ml/kg. Ventilator settings and pO_2 were adjusted to maintain arterial blood gas parameters within the physiological range. The left jugular vein was cannulated with polyethylene tube for continuous infusion of 0.9% saline. The right carotid artery was cannulated with a cannula filled with heparinized saline and connected via pressure transducer to CARDIOSYSCO-10l (Experimentria, Hungary) for the measurement of blood pressure and heart rate. A left thoracotomy was preformed through the fifth intercostal space and the heart was exposed. A wide bore (1.5 mm) sterile metal cannula connected to a pressure transducer (Gould Statham P23ID, USA) was inserted into the cavity of left ventricle from the posterior apical region of heart for recording left ventricular pressure dynamics on polygraph (Grass 7D, USA). After the completion of surgical procedures, the thoracic cavity was covered with saline soaked gauze to prevent the heart from drying. The animals were then allowed to stabilize for 10 min before recording the basal hemodynamic variables.

2.6. Assessment of biochemical parameters

Heart samples were removed from liquid nitrogen and brought to room temperature and weighed. A 10% homogenate of myocardial tissue was prepared in 50 mM phosphate buffer (pH 7.4) and an aliquot was used for the assay of thiobarbituric acid reactive substances (TBARS), according to the method described by Ohkawa et al. (1979). Protein free supernatant obtained by the addition of equal volume of 10% TCA to the tissue homogenate and centrifuging at 5000 rpm for 10 min, was used for the estimation of reduced glutathione (GSH) (Moron et al., 1979). The tissue homogenate was centrifuged at 8000 rpm for 30 min at 4 °C and the supernatant obtained was used for the estimation of other biochemical parameters: lactate dehydrogenase (LDH) (Cabaud and Wroblewski, 1958), superoxide dismutase (SOD) (Marklund and Marklund, 1974) and catalase (CAT) (Aebi, 1984). Creatine kinase-MB (CK-MB) isoenzyme was estimated spectrophotometrically using a kit from Spinreact, Spain.