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Cardiaprotective effect of crocetin by attenuating apoptosis in isoproterenol induced myocardial infarction rat model

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ABSTRACT

Given study evaluates the cardioprotective effect of crocetin in myocardial infracted (MI) rats. MI was produced by administering isoproterenol (90 mg/kg/day, i.p.) in rats for two consecutive days. all the animals were divided in to four groups such as control group receives only saline; MI group which receives only isoproterenol and crocetin treated group which receives crocetin (50, 100 and 200 mg/kg/day, p.o.) for the duration of 15 days. At the end of dosing left ventricular functions was assessed to estimate its effect on cardiac functions. Catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), creatine kinase (CK-MB), lactate dehydrogenase (LDH) and inflammatory cytokines were determined in the cardiac tissue homogenate. Histopathology study was also carried out using hematoxylin and eosin staining. Immunohistochemistry was done for the estimation of Caspase-3, Bcl-2, Bax and Nrf-2 level in the myocardial tissues of MI rats.

Result of the study suggested that GSH, CAT, CK-MB, and LDH were ($p < 0.01$) increased in the tissue homogenate of crocetin treated group than MI group. However crocetin significantly ($p < 0.01$) decreases the level of MDA and activity of SOD in the tissue homogenate than MI group. It was observed that treatment with crocetin attenuates the level of inflammatory cytokines in the myocardial tissues of MI rats. Moreover level of caspase-3, Bax and Nrf-2 significantly reduced and Bcl-2 enhanced in the myocardial tissues of MI rats than MI group. The altered cellular architecture of heart tissue sections in the myocardial infracted rats were reversed by administration of crocetin treatment. Taking all these data together, it may be suggested that the crocetin act as a possible protective agent in myocardial infarction by decreasing oxidative stress and inflammatory cytokines and thereby attenuates the apoptosis of myocardial cells.

1. Introduction

Myocardial infarction defined as a condition of acute myocardial necrosis. It occurs due to difference and imbalance between myocardial demand and blood supply [1]. Myocardial infarction leads to hyperlipidemia, lipid peroxidation, hyperglycemia and free radical damage. Prevention of damage and restoration of blood supply to the ischemic tissue can be a possible treatment for the ischemic injury. Formation of lipid peroxides, defective antioxidant system, and cell membrane destruction occurs due to the formation of free radicals such as hydroxyl radicals and superoxide anion [2,3]. Suppression of free radical formation and endogenous antioxidant enzymes development could reduce the infarct size of the injured myocardial region [4].

Myocardial infarction has been induced by the administration of isoproterenol. It is non-selective β adrenoceptor agonist and synthetic. It produces physiological disturbances between antioxidant defense mechanism and free radical formation [5,6]. Altered antioxidant enzymes and increased lipid peroxidation have been observed in acute myocardial necrosis [7]. Isoproterenol-induced myocardial infarction in rats has been found same to morphological and pathophysiological characteristics of human myocardial infarction [8].

Crocetin is the active and key constituent of saffron (*Crocus sativus* L) and pharmacologically active [9]. Saffron and its constituents have been reported to have anticonvulsant [10], antidepressant [11], bronchodilator [12] and anti-tumor effect [13]. Also, crocetin has been reported to reduce lipid peroxidation [14], muscle-skeletal and hippocampal in ischemia-reperfusion-

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induced oxidative damage in male albino rats [15,16]. The free radical scavenging activity of crocetin has been reported using DPPH [17]. Therefore, the present study investigated the protective effect of crocetin on cardiac tissue in myocardial infarction.

2. Materials and methods

2.1. Animals

Male wistar rats (200–220 g) were used in this study. All the animals were maintained under the standard condition given as per the ethical guidelines. All the experiments used in the given study were approved by institutional ethical committee of First Affiliated Hospital of Xi'an Jiaotong University, China.

2.2. Chemicals

All the chemical reagents and biochemical used in the study were of analytical grade and of highest purity. The enzyme standards, isoproterenol and crocetin used in this study were procured from Sigma Aldrich., St. Louis, USA.

2.3. Experimentation

Myocardial infarction was produced by i.p. injection of isoproterenol (90 mg/kg/day) for two consecutive days. Later all the animals were divided in to four groups such as control group receives only saline; MI group which receives only isoproterenol and crocetin treated group which receives crocetin (50, 100 and 200 mg/kg/day, p.o.) for the duration of 15 days.

2.4. Determination of left ventricular and hemodynamic

All the animals were anesthetized by pentobarbitone (60 mg/kg, i.p.) and 0.1 mg/kg of atropine was given i.p. for the maintenance of heart rate. Method reported by Ojha et al. was used for the surgery for the estimation of hemodynamic parameters [18].

2.5. Determination of biochemical parameters

All the animals were sacrificed by cervical dislocation and heart was isolated from each animals. Phosphate buffer was used to homogenate the myocardial tissues and this homogenate was used for the estimation GSH and MDA [19,20]. Moreover other oxidative stress parameters (SOD and catalase) [21,22] and LDH, CK-MB and total protein [23] were also estimated in the tissue homogenate.

2.6. Estimation of cytokines

The level of cytokines such as IL1β, IL6 and TNFα were estimated in the tissue homogenate by using specific enzyme-linked immunosorbent assay (ELISA) kits and detailed procedure used as per the instruction given in individual kit.

2.7. Histopathology study

Method explained by Ojha et al., was used to study histopathology of cardiac tissues. Isolated heart was kept in formalin solution to fix it. Later added it in to liquid paraffin and prepare wax cubes of it. Sections of tissue were cut by the help of microtome of 4 μm size and fix it on the slide. hematoxylin-eosin stain was used to stain the tissue and trinocular microscope was used to characterize the tissue [18].

2.8. Immunohistochemistry

Isolated cardiac tissues were fixed in paraffin sections and cut in to tissue thickness (5 μm). Tissues were dehydrated with ethanol after deparaffinized by xylene and tissue sections were incubated for 5 min at 98 °C with antigen in PBS solution. PBS solution was used to wash tissue section and then incubate for 10 min with 3% H_2O_2 at room temperature. Thereafter primary antibody anti-Caspase-3 (1:200), anti-Bcl-2 (1:250), anti-Bax (1:200) and anti-

Table 1
Assessment of crocetin effect on Left Ventricular function in myocardial infracted rat.

Sr. No.	Group	+LVdP/dtmax (mm of Hg/s)	-LVdP/dtmax (mm of Hg/s)	LVEDP (mm of Hg)
1	Control	3530 ± 102	3291 ± 174	3.85 ± 0.64
2	MI	2064 ± 42**	1875 ± 81**	8.72 ± 1.09**
3	Crocetin 50 mg/kg	2305 ± 57#	2150 ± 95##	7.90 ± 0.81##
4	Crocetin 100 mg/kg	2863 ± 71##	2783 ± 126##	6.25 ± 0.59##
5	Crocetin 200 mg/kg	3286 ± 94##	3158 ± 105##	4.38 ± 0.71##

Values expressed as Mean ± SEM (n = 10).

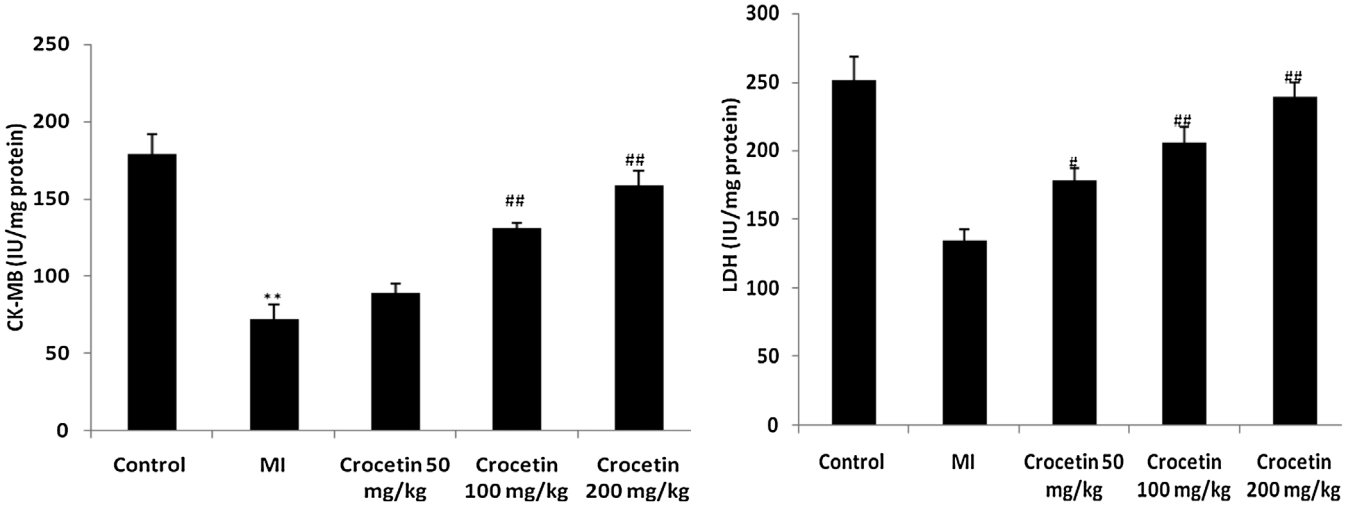

Fig. 1. Assessment of crocetin effect on the markers of myocardial infarction in cardiac tissue.