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The Effect of Saffron Consumption on Biochemical and Histopathological Heart Indices of Rats with Myocardial Infarction

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Abstract This study was designed to assess the effects of saffron (Crocus sativus) on rats' heart with isoproterenol-induced myocardial injury. Animals were divided randomly into four groups: vehicle-control group (CTL); ISO group, administrated with Isoproterenol 85 mg/kg s.c.; saffron group; and finally combined Saffron + ISO group. Basal and final serum levels of heart troponin I, heart tissue antioxidants and histopathological indices were assessed in all groups. Isoproterenol administration significantly increased serum level of troponin I when compared to control group (3.46 ± 0.77 vs. 0.53 ± 0.35 ml in ng/ml, $P < 0.001$) and reduced significantly the glutathione peroxidase activity of heart muscle (1.63 ± 0.21 vs. 4.01 ± 0.64 nmol/mg protein, $P < 0.05$). The grade of heart muscle damages was severe in more than 70% of ISO group animals. Saffron + ISO group showed remarkably decreased intensity of tissue destruction and significantly decreased serum levels of heart troponin I, when compared to ISO group (1.25 ± 0.23 vs. 3.46 ± 0.77 ng/ml, $P < 0.05$). The level of glutathione peroxidase activity in Saffron + ISO animals did not have significant decline compared to saffron alone. These results suggest the protective role of saffron on ischemic hearts by biochemical and histopathological findings.

Keywords Saffron · Myocardial injury · Cardioprotective · Antioxidant · Histopathological indices

Introduction

The dried stigmas of *Crocus sativus* L. (saffron) are used as a food seasoning and colorant and in folk medicine for therapeutic purposes. This pretty spice is a common additive to fish, rice and other dishes in several cuisines of different cultures such as Iranian, Spanish, French, Italian, Arabic, Indian and Mediterranean countries [1].

Antispasmodic, expectorant, anticatarrhal, stimulant, stomachic, eupeptic, aphrodisiac and nerve sedative are some of the saffron properties, which have been described in folk medicine [2]. Recent studies have demonstrated the antinociceptive and antiinflammatory [3], antitumor effect [4], learning and memory improving [5], hypotensive [6] as well as calcium channel inhibitory [7] properties of saffron. Hosseinzadeh et al. showed that the aqueous saffron extract (*Crocus sativus* L.) and its active constituent, crocin, have protective effect on renal ischemia–reperfusion injury, which was induced by oxidative stress in rats [8]. Neuroprotective effects of saffron and some of its constituents have been demonstrated by other experimental studies [9, 10]. In spite of saffron utilization as a spice in many societies, no study in literature has yet dealt with the effects of saffron consumption on ischemic heart.

Thus, the aim of present study was to assess the effects of saffron consumption on tissue levels of antioxidants,

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biochemical and histopathological indices for normal and ischemic rat hearts.

Materials and Methods

Experiments were conformed to national guidelines for conducting animal studies (Ethic committee permission No 86/123KA—Kerman University of Medical Sciences) and performed on 38 male Wistar rats aged 3 months and weighed 250–300 g.

Chemicals

Isoproterenol was purchased from Sigma (England), sodium thiopental from Biochemie (Austria), SOD assay kit-WST from Dojindo Molecular Technologies, Inc. (USA), GPX assay kit from Randox Laboratory Ltd. (UK) and troponin I assay kit from BioMerieux (France).

Preparation of Saffron

Saffron (*Crocus sativus* L.) stigmas were collected from Zarand (Kerman province, Iran), identified and confirmed by Botany Department of Bahonar, University of Kerman, Iran. Dried stigmas were chopped and macerated in tap water for 3 days. Then the mixture was filtered and prepared solution included 100 mg/ml concentration from dry weight of stigma. This method was chosen based on the current using pattern among consumers.

Animals Groups

Thirty-eight male Wistar rats weighing 250–300 g were divided randomly into four groups of CTL (vehicle-control), ISO, Saffron and Saffron + ISO groups. In CTL, animals received 1 cc/kg of tap water by gastric tube for 7 days and 1 cc/kg normal saline injected subcutaneously daily in final 2 days (days of 6 and 7) of experiment. Subjects in ISO group received 1 cc/kg of tap water similar to CTL group and administrated with isoproterenol 85 mg/kg daily in final 2 days of experiment for cardiac injury induction [11]. Saffron group was fed with saffron solution, the similar volume of tap water used for aforementioned groups, which is equivalent to 100 mg/kg of dry weight stigma. This dose was selected based on previous experimental studies which have shown that 100 mg/kg of saffron per day is more efficacious and without any recognized side effects [12–14]. Finally, the Saffron + ISO group received saffron solution and isoproterenol similar to Saffron and ISO groups, respectively.

Biochemical Measurements

In all groups, blood sample was taken at the start and termination of experiments for basal and final troponin I assay. After centrifuging the blood, the serum was separated and stored at −20°C for the maximum of 2 weeks until troponin I was measured by VIDAS troponin I ultra assay, which is an enzyme-linked fluorescent immunoassay-based method by relative Kit. At the end of experiments, the animals were anaesthetized with sodium thiopental (50 mg/kg i.p.), then killed, hearts were removed, washed with cold saline and a piece of heart apex dissected, weighted and homogenized in 5 ml of 0.1 M Tris–HCl buffer (pH 7.4) in ice-cold condition. After centrifuging, the clear supernatant solution was taken for the biochemical analysis. Total proteins measured by using the Lowry et al. method [15]. Malondialdehyde (MDA) levels, an index of lipid peroxidation which produced by oxidative elements activation, were estimated by concentration of thiobarbituric acid reactive substances (TBARS) in heart tissue [16]. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity in tissues were determined using the SOD assay kit-WST and Randox assay kit, respectively (according to the manufacturer's protocol) [17].

Histopathology

The heart tissues were fixed using 10% buffered formalin and embedded in paraffin. Thereafter, 5-μm thick paraffin sections were prepared from samples, stained with hematoxylin and eosin (H&E), and examined microscopically by two pathologists blind-folded of animal grouping. The slides were evaluated under light microscope (Nikon, Tokyo, Japan) fitted with digital camera and the lesions graded as (0) nil; (1) minimum (focal myocytes damage); (2) mild (small multifocal degeneration with slight degree of inflammatory process); (3) moderate (extensive myofibrillar degeneration and/or diffuse inflammatory process); (4) severe (necrosis with diffuse inflammatory process) [11].

Statistical Analysis

Values are presented as the Mean ± SEM. Comparisons were performed between basal and final values in each group by student paired *t*-test and among different groups by one-way ANOVA followed by post hoc Tukey's test. Differences in the histopathological scores were determined by chi square and Fisher's exact tests. *P*-value <0.05 was considered as statistically significant.

Results

Plasma Cardiac Troponin I Levels

The final Plasma cardiac troponin I levels were increased significantly in groups with cardiac ischemia, ISO and Saffron + ISO groups, compared to basal levels which was more remarkable in ISO groups ($P < 0.001$ and $P < 0.01$, respectively; Fig. 1). Comparisons among groups showed significant increase of final troponin I levels only in ISO group, when compared to CTL group ($P < 0.001$), whereas, there was decreased significance in Saffron + ISO group compared to ISO group ($P < 0.05$; Fig. 1).

MDA Tissue Levels

The level of MDA in heart tissues of animals which have been administered isoproterenol alone was higher, but this difference was not statistically significant (Fig. 2).

GPX Tissue Activity

GPX tissue activity declined significantly in ISO group when compared to CTL group ($P < 0.05$), while pre-treatment with saffron feeding modulated this decline as it was not significant between Saffron + ISO group and CTL group and relative CTL i.e. saffron group. GPX tissue activity showed nonsignificant higher levels in Saffron + ISO group when compared with ISO group alone (Fig. 3).

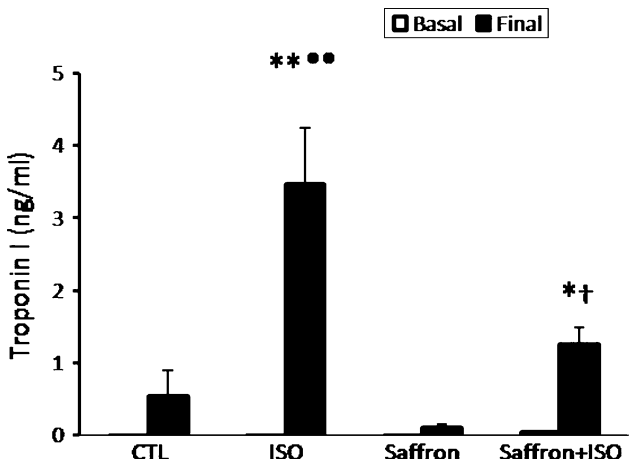

Fig. 1 Basal and final serum levels of troponin I in different animal groups. Values are means ± SE. $n = 8$–11; *CTL* control, *ISO* isoproterenol. * $P < 0.01$ and ** $P < 0.001$ compared with relative basal level. •• $P < 0.001$ compared to CTL group. † $P < 0.05$ compared to saffron group

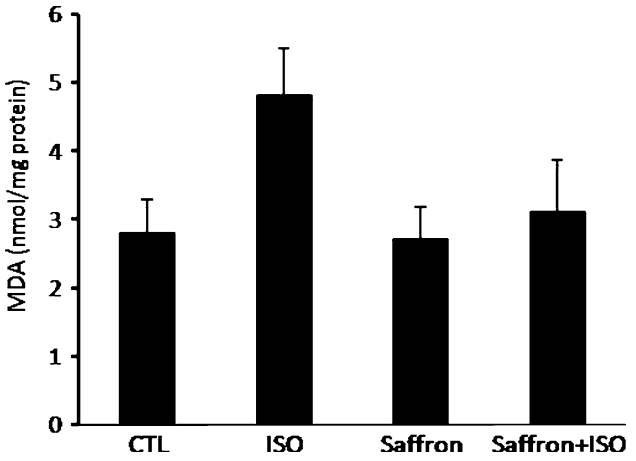

Fig. 2 Cardiac tissue levels of MDA (*Malondialdehyde*) in different animal groups. Values are means ± SE. $n = 8$–11; *CTL* control, *ISO* isoproterenol

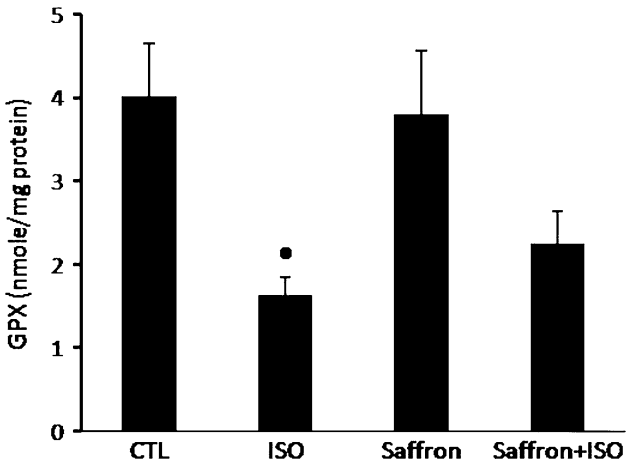

Fig. 3 GPX (*Glutathione peroxidase*) tissue activity in different animal groups. Values are means ± SE. $n = 8$–11; *CTL* control, *ISO* isoproterenol. • $P < 0.05$ compared to CTL group

SOD Tissue Activity

Activity of SOD enzyme in heart tissue decreased in groups' endured myocardial ischemia, ISO and Saffron + ISO. But it was not statistically significant when compared to corresponding control groups (Fig. 4).

Histopathological Findings

Statistical comparison of histopathological scores showed significant differences among animal groups ($P < 0.001$). The highest cardiac myodegeneration was observed in myocardial tissue of ISO group (72.7% of these animals). Pre-treatment with saffron modulated the myocardial degeneration effects of isoprotrenol, as it has been shown in Table 1 and Fig. 5. Most of outstanding pathological findings in Saffron + ISO group were small multifocal

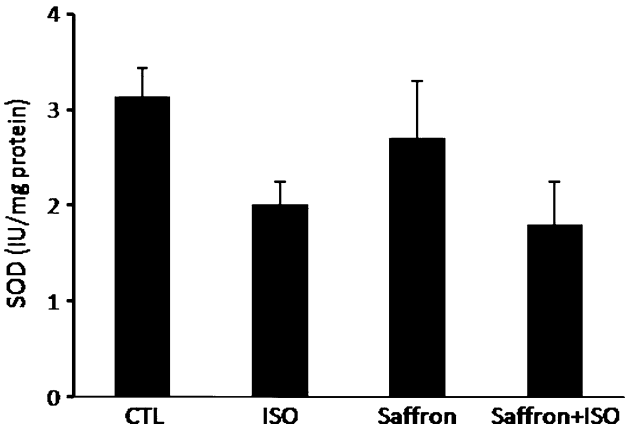

Fig. 4 SOD (*Superoxide dismutase*) tissue activity in different animal groups. Values are means ± SE. $n = 8–11$; *CTL* control, *ISO* isoproterenol

Table 1 Pathology score in different animal groups

Pathology score		Group				Total
		CTL	ISO	SAF	SAF + ISO	
0	Count	10	0	8	2	20
	% Within group	100.0	.0	100.0	22.2	52.6
1	Count	0	0	0	2	2
	% Within group	.0	.0	.0	22.2	5.3
2	Count	0	2	0	4	6
	% Within group	.0	18.2	.0	44.4	15.8
3	Count	0	1	0	1	2
	% Within group	.0	9.1	.0	11.1	5.3
4	Count	0	8	0	0	8
	% Within group	.0	72.7	.0	.0	21.1
Total	Count	10	11	8	9	38

CTL control, *ISO* isoproterenol, *SAF* Saffron, pathology scores: *0* nil, *1* minimum (focal myocytes damage), *2* mild (small multifocal degeneration with slight degree of inflammatory process), *3* moderate (extensive myofibrillar degeneration and/or diffuse inflammatory process), *4* severe (necrosis with diffuse inflammatory process)

degeneration with slight degree of inflammatory process (44.4% of group's subjects) in subendocardial area but without any sever tissue destruction. Moreover, there was intact myocardial tissue in saffron group (Table 1 and Fig. 5).

Discussion

The aim of present study was to determine the effects of saffron consumption on heart endurance in confronting the ischemic stressful conditions. The results indicated that saffron consumption modulates the destructive effects of isoproterenol on myocardial tissue notably by stabilizing effects on heart muscle antioxidant activities.

It has well been known that high doses of isoproterenol, a nonselective beta adrenergic agonist, induces cardiotoxicity and cardiac damages (myocardial infarction-like lesions), as a result of increasing contractility and heart rate, hypotension and imbalance between oxygen supply/demand of heart [18, 19]. These processes are associated with increase in serum cardiac troponin levels, which closely correlated with the severity of myocardial damage [19]. Moreover, it has been suggested that isoproterenol through auto-oxidation of catecholamines alters the reactive oxygen species (ROS)/antioxidative defense system balance in favor of more free radicals production, which leads to oxidative damage of cardiac cells [20, 21]. Our findings showed high increases in cardiac troponin I levels, decreasing pattern in antioxidant enzyme activity, nonsignificant increasing trend of MDA and sever myocardial injury in animals, which was administered isoproterenol for 2 days. These results are in accordance with previous investigations. Reduced GPx activity was significant in ISO group. This enzyme has a protective role for cellular and subcellular membranes from the peroxidative injury by eliminating hydrogen peroxide and lipid peroxides [22]. Therefore, decreased activities of this enzyme resulted in the aggregation of these oxidants, and myocardial cell membranes will be more vulnerable to free radicals damage [23]. GPx activity was recovered in animal's group, which was pre-treated with saffron, saffron + ISO. Previous investigations suggested that some constituents of saffron might have antioxidant properties [4]. Ochiai et al. and Saleem et al. in two independent studies showed protective roles for saffron and its carotenoid components on neuronal cell injuries in vivo and in vitro [9, 12]. In another experiment, Hosseinzadeh et al. demonstrated that saffron extract and its constituent, crocin, have protective effect on ischemia reperfusion (IR)-induced oxidative stress in rat kidney [8].

All of these researchers in line with our study have proposed an antioxidant role for saffron. Therefore, it has been suggested that at least one part of cardioprotection effects of saffron is associated with antioxidant activity of the spice. Saffron consumption prevented cardiac injury from developing and decreased serum levels of cardiac troponin I. Boskabady et al. documented that aqueous-ethanol extract of saffron has a potent inhibitory effect on the calcium channel of guinea-pig isolated heart [7]. As mentioned thus far, isoproterenol has positive inotropic and chronotropic effects, which produce coronary hypotension and markedly increases the workload and oxygen demand of myocardial muscle. In addition, these effects lead to cytosolic calcium overload that mediated through calcium channel, which in turn increases heart susceptibility to

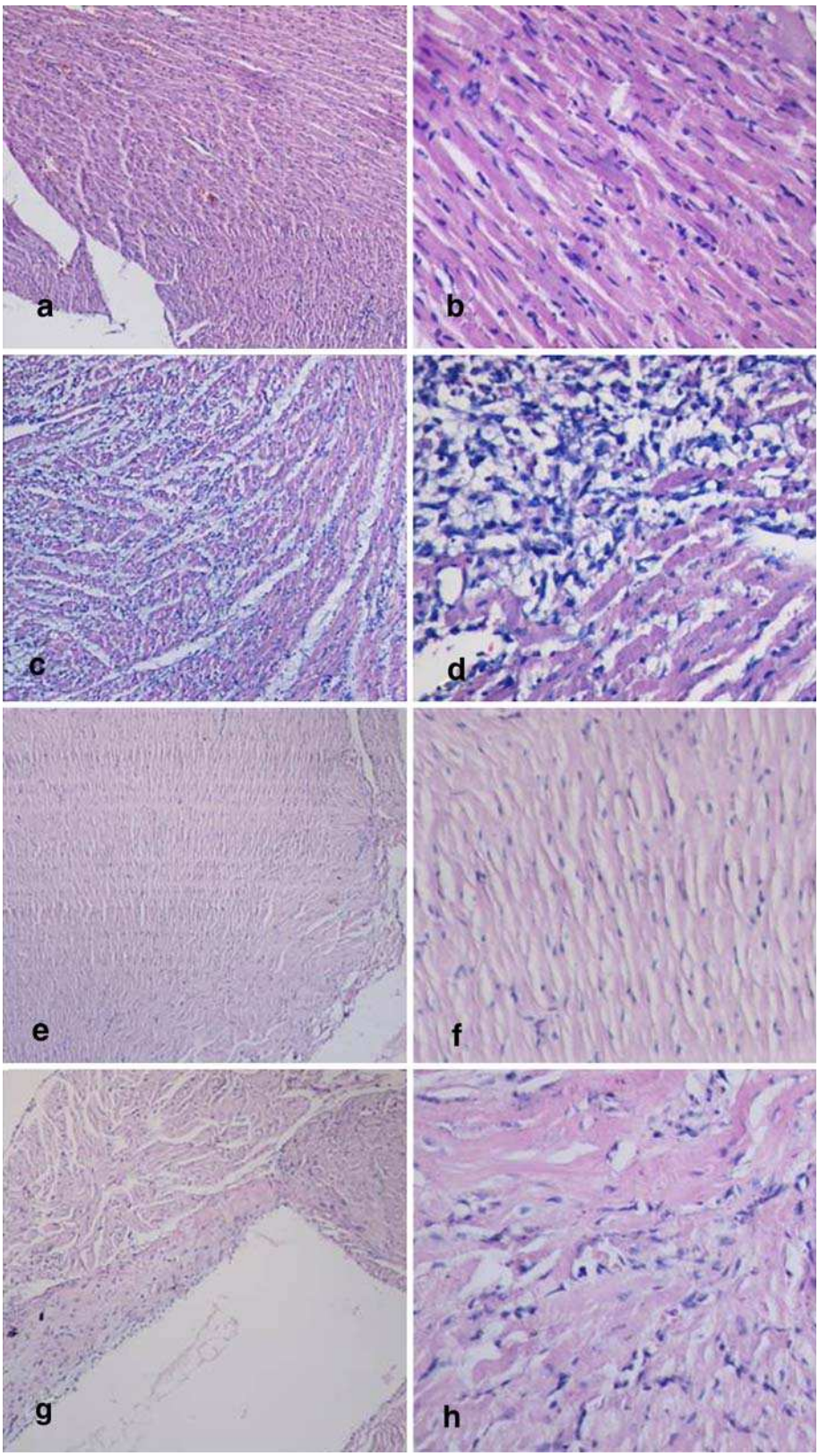

Fig. 5 H & E stained sections of heart tissue in different animal groups. The magnification of **a**, **c**, **e** and **g** is ×100 and others are ×400. **a**, **b** CTL group heart sections showing normal appearance of cardiac myofibers. **c**, **d** ISO group sections showing necrosis of muscle fibers, diffuse inflammatory cell infiltration, edema and fibroblastic proliferation. **e**, **f** Normal architecture of myocytes in saffron group. **g**, **h** Small multifocal degeneration, edema and slight degree of inflammatory process in subendocardial area of Saffron + ISO group

injury [24]. Therefore, another supposed mechanism for cardioprotective effect of saffron is negative inotropic and chronotropic effects, which are being enforced by cardiac calcium channels inhibition. Calcium channel blocking decreases heart rate and contractility, and finally reduces heart workload and prevents heart from injury.

In conclusion, this study suggested that oral consumption of saffron, routine form of usage in public, has cardioprotective effects on heart, which partly is exerted by stability and even amplification of antioxidant system and also, it is possible by decreasing heart rate and contractility in stressful conditions. However, supplementary studies are required for clarifying the entire aspects of saffron effects on heart.

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