Dietary saffron reduced the blood pressure and prevented remodeling of the aorta in L-NAME-induced hypertensive rats

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Introduction

*Crocus sativus L.* commonly known as saffron, is a fragrant plant that belongs to the Iridaceae family. It is cultivated in several countries including Iran. Saffron has been used in traditional medicine for several purposes (1), as it has many pharmacological effects including antioxidant (2), analgesic, anti-inflammatory, anticonvulsant, antianxiety, aphrodisiac, antidepressant, (1) and bronchodilator activity (3).

In addition, saffron and its constituents have cardiovascular properties that include hypotensive (4) and cardioprotective effects in rats (5), platelet aggregation inhibition and membrane lipid peroxidation in humans (6), and antiatherosclerosis effects in rabbits (7). Furthermore, it has been reported that chronic IP administration of saffron stigma aqueous extract and its major components: crocin and safranal reduced systolic blood pressure (BP) of desoxycorticosterone acetate (DOCA)-salt induced hypertensive rats (8-10).

Nitric oxide (NO) which plays an important role in maintaining normal BP and essential hypertension is associated with defects in the NO. Chronic administration of NO synthase (NOS) inhibitor, nitro-L-arginine methyl ester (L-NAME), caused a significant increase in BP of experimental animal, a particularly interesting model of hypertension which looks like essential hypertension model (11) that was used in this study.

Saffron is used extensively as a food additive in Iran and other countries, but its dietary effects on BP and aorta remodeling in hypertensive rat are not well known. So, the aim of this study was to investigate the nutritional effects of saffron stigma hydroalcoholic extract on BP and histological changes of the thoracic aorta in normotensive and L-NAME induced hypertensive rats.

Materials and Methods

**Animals and drugs**

The experimental protocol was approved by the Ethical Review Board of Semnan University of Medical Sciences, Iran (license number: 91.7861). All experimental trials were conducted in agreement with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Male Wistar rats were obtained from the breeding colony of Semnan University of Medical Sciences, Semnan, Iran.

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Iran. Animals were housed in individual cages in a 12-hr light/dark cycle at 22–24 °C, with food and water available ad libitum.

The drugs used in this study including thiopental sodium and L-NAME, were obtained from Sigma-Aldrich; heparin was obtained from TRITTAU, Germany. Drugs and extracts were dissolved in saline.

**Experimental procedure**

**Nutritional effects of Saffron on BP**

To determine chronic effects (dietary) of saffron on BP and the histomorphometry of the aorta in hypertensive and normotensive rats, 28 male Wistar rats (180–220 g) were divided randomly and equally into the following four experimental groups: (1) L-NAME plus vehicle, (2) L-NAME plus saffron, (3) vehicle plus vehicle, and (4) vehicle plus saffron. Hypertension was induced by L-NAME (40 mg/kg/day) administration through drinking water for 5 weeks (11); saffron (200 mg/kg/day) or its vehicle was given orally during this period. BP was measured noninvasively weekly with the cuff method (Power lab-Australia) and invasively at the final step of experiment.

**Histomorphometry**

Rats in the chronic groups and normal group were sacrificed under deep anesthesia at the end of the experiment. The thoracic aorta segments were removed and fixed in 10% formalin solution for 48 hr. The samples were embedded in paraffin and handled with routine tissue processing. The 5 μm sections were stained by hematoxylin and eosin (H&E), orcein, and periodic acid Schiff (PAS) methods. Magnified digital images of the stained aortic slices were obtained with a digital camera (Nikon, CoolPix S10, Japan) attached to a light microscope (Zeiss, Germany). All slides were evaluated using a light microscope, which was calibrated by an ocular micrometer. The aortic cross-sectional area, aortic wall thickness, tunica media thickness (from the internal to the external elastic lamellae), and number and thickness of elastic lamellae were calculated in ten areas of five random sections of the thoracic aorta obtained from each animal.

**Plant and extract preparation**

Saffron stigma (*Crocus sativus* L.) was prepared from Ghaen city in Khorasan Province, Iran, in the fall of 2012 and certified by botanists in the Organization of Promotion, Education, and Agricultural Research-Semnan. Saffron stigma was ground, and 10 g of its powder was added to 1000 ml of 70% ethanol. The mixture was warmed to 50 °C while mixing on a hotplate stirrer (Sana-Iran) for about 30 min. The solution was filtered, dried by oven at 40 °C, and stored in refrigerator. The yield of the extract was about 30%. The dried extract was dissolved in saline daily to make different doses of saffron.

**Statistical analysis**

The results are presented as the mean±SEM; a P-value<0.05 was accepted as statistically significant. The paired t-test was used for within group comparisons, and one-way analysis of variance (ANOVA) was used for between group comparisons, which was followed by the Holm–Sidak method. When the data failed the normality test, ANOVA on ranks (Kruskal–Wallis) was used, followed by Dunn’s method for multiple comparisons (SigmaStat.3.0).

**Results**

**Dietary effects of saffron on BP of normotensive and hypertensive rats**

Hypertension induction significantly increased systolic BP from 123.3 to 176.14 mm Hg. Dietary saffron prevented L-NAME-induced increases in the systolic BP of hypertensive rats. This effect was clearly observed at the third week of treatment (Figure 1). Saffron and its vehicle had no effect on the systolic BP of normal rats. BP values recorded noninvasively at the end of the experiment were consistent with those recorded weekly with the noninvasive method.

**Dietary effect of saffron on the histomorphometry of aorta in normotensive and hypertensive rats**

The tunical layer of the thoracic aorta (tunica intima, media, and adventitia) in control animals showed normal histological features. The tunica media was observed in numerous distinct lamellae, which were wavy and arranged concentrically (Figure 2a). In hypertensive rats, the aortic cross-sectional area, aortic wall thickness, and tunica media thickness were increased. The aortic wall thickening was mainly due to thickening of the tunica media. The number and thickness of concentric elastic lamellae were also increased in the tunica media (Figures 2b and 2c). Saffron extract administration (200 mg/kg/day) caused a significant reduction in the aortic cross-sectional area, the tunica media thickness, and the number of elastic lamellae in hypertensive rats (Figures 2d and 2e).

![Figure 1. Chronic nutritional effects of saffron extract (200 mg/kg/day) on systolic blood pressure (SBP) of normal and L-NAME-induced hypertensive rats during 5 weeks (*P<0.001). H-V: hypertensive + vehicle, H-S: hypertensive + saffron, N-V: Normal + vehicle, N-S: Normal + saffron, W0-5: weeks of 0-5](image-url)
Antioxidant deficit is a basic factor in hypertensive animals. Reactive oxygen species (ROS) play a role in vascular remodeling (proliferation, migration, and hypertrophy), and consequently, in the pathology of atherosclerosis and hypertension (11). As saffron has antioxidant activity (2, 15) it might affect L-NAME-induced hypertension. Moreover, safranal acts on GABAA receptors that modulate peripheral resistance or cardiac output, which also might contribute to its hypotensive effect (4).

Hypertension induces numerous pathological changes in the cardiovascular system. Histological examination of the aorta revealed that hypertension increased the aortic area, aortic wall and media thickness, and elastic lamellae number and thickness. Saffron treatment significantly reduced most of these factors, except for aortic wall and elastic lamellae thickness. The beneficial effects of dietary saffron might induce antioxidant activity or reduce BP; however, further clarification is necessary. No change in BP or histological factors has been considered in normal rats.

In summary, the present study provides evidence that nutritional saffron prevents BP increases and aortic remodeling in the L-NAME induced hypertensive rat. These findings suggest that saffron might be useful in hypertension.

Acknowledgment
This study was supported by a grant from Semnan University of Medical Sciences, Semnan, Iran. We are most grateful from Vice Chancellor for Research Centers, Heads of Departments of Physiology and Anatomical Sciences for support and aids. The results described in this paper were part of a student thesis.

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