Anti-Atherosclerotic Effects of the Hydroalcoholic Extract of *Crocus sativus L.* (saffron) Petals on Hypercholesterolemic Rats

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ABSTRACT

Cardiovascular diseases are the major causes of mortalities worldwide. One of the methods for preventing and fighting cardiovascular diseases is the use of medicinal herbs that have been known to have fewer side effects as compared to chemical drugs. The present study aimed at investigating the effects of the hydroalcoholic extract of saffron (Crocus Sativus L.) petals on the atherosclerotic plaques. For this purpose, male Wistar Rats with the average weights of 170-220 g were assigned to six 5-member groups. The treatments lasted for 6 weeks. To perform histological examinations, the aorta and hearts of the rats were dissected at the end of the 6th week, and kept in a 10% formalin solution. The aorta tissues in the control group were fed with an ordinary diet and were thus completely healthy and natural, but atherogenic lesions and plaque formation symptoms were evidenced in the sham group that had been fed with a high-cholesterol diet. No signs of tissue or atherogenic lesions were detected in the 4 experimental groups treated with saffron petal extract plus lovastatin at various dosages. Also, a significant difference was found between the average weights of all the groups and the sham group ($P<0.001$). The hydroalcoholic extract of saffron petals was interestingly found to act like lovastatin, which is a chemical drug for preventing atherogenic lesions. Compared to chemical drugs, medicinal herbs as a source of different antioxidants can provide an appropriate alternative mitigation of cardiovascular diseases.

Key Words: Cardiovascular diseases, *Crocus Sativus L.*, Hydroalcoholic extract, Atherosclerotic plaques, Rats.

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INTRODUCTION

About 30% and 38% of the mortalities around the globe and in Iran have been known to be caused by cardiovascular diseases. According to some experts' declarations, it is nearly 3000 years that people have lost their useful lives because of the mentioned diseases [1, 2]. Hypertension and atherosclerosis are the most prevalent symptoms and risk factors of cardiovascular diseases [3, 4].

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Nowadays, the use of chemical drugs has become inevitable. However, researchers are seeking to find more effective drugs primarily extracted from medicinal herbs to treat diseases because of having fewer side effects. According to the rich background of traditional medicine and its underlying use of medicinal herbs for treating diseases in Iran, performing extensive scientific research for identifying their pharmaceutical and therapeutic effects and determining their efficient consumption levels is incumbent. Saffron, which is scientifically called Crocus Sativus L., belongs to the plant family of Iridacea. It is one of the major plants cultivated and grown in Iran. There are numerous references to its many pharmaceutical features. According to the related studies, saffron petal contains glyconfalavonolmyristine, crocetin, Kaempferol, and two types of anthocyanin called delphinidin and petunidin [5]. The most significant color agents in saffron are carotenoids, including crocetin and crocin. Besides being applied as an essence in various industries, saffron possesses abundant pharmaceutical and therapeutic properties [6]. Therefore, various pharmacological studies have been indicative of abundant applications of saffron and its active ingredients; the most important of which are anti-depression [7], anti-anxiety and soporific [8], anti-convulsion [9], anti-hypertension [10], analgesic and anti-inflammatory [11], anti-tumor [12], and anti-coughing effects [13], influence on the morphine withdrawal syndrome [14], as well as the cellular and humoral immunity [15] and an array of the other influences including moderating the blood sugar and lipid [16], delaying alzheimer [17], relaxing muscles [18] and improving memory and learning [19].

Due to its many useful effects and active ingredients, the present study dealt with the investigation of what effects the hydroalcoholic extract of saffron petals has on atherosclerotic plaques.

METHODS

Fresh saffron petals were collected from the farms of Boshrouyeh City in South Khorasan Province, dried in the shade, and milled in the Phytobiology Department, Basic Sciences Faculty of Lorestan University after performing its scientific identification and verification. The petals were placed in a hydroalcoholic solvent containing a water/ethanol ratio of 20/80 for 3 days and then, the solution was stirred in a shaker device. To filter the extract, Whatman filter paper was utilized. The filtered solution was condensed in a rotary device and then placed in a 30-degree-centigrade oven. The dried plates were scraped by the use of spatula after a period of 24 hours, and the residues were kept in 4-degree-centigrade dark glass containers to undergo further experiments [20].

Male Wistar rats in a weight range of 170-200 g were procured from Razi Serum Institute, kept in a laboratory belonging to the Faculty of Medicine, Ilam University of Medical Sciences at a temperature range of 20-23°C and under a 12-h light/dark cycle, and then fed with tap water and ordinary rodent food. Afterwards, they were randomly assigned to six 5-member groups as follows:

Control Group: an ordinary food diet + normal saline.

Sham Group: a high cholesterol diet (2%) + normal saline.

Experimental Group One: a high cholesterol diet (2%) + an extract in a dosage of 50 mg/kg body weight per day.

Experimental Group Two: a high cholesterol diet (2%) + an extract in a dosage of 100 mg/kg body weight per day.

Experimental Group Three: a high cholesterol diet (2%) + an extract in a dosage of 200 mg/kg body weight per day.

Experimental Group Four: a high cholesterol diet (2%) + lovastatin in a dosage of 10 mg/kg body weight per day.

The hydroalcoholic extract of saffron petals was administered to the animals of different groups through oral gavage. They received their specified dosages of the extract using a disposable gavage needle exactly at the same hours every day. The treatments lasted for 4 weeks.

The weights of all the animals were measured and recorded during the 6-week period. At the end of the 6th week, they were anesthetized by ether according to the ethical principles of research on laboratory animals and subsequently undergone systematic autopsies. Their hearts and aortas were dissected from a sterile area and then placed in a 10% buffer solution to be stained with hematoxylin and eosin (H&E) for 24 hours. The stained slides of the tissues were prepared and light microscopy was applied for histological examinations. The results of their weights were also analyzed using SPSS (ver. 16) and the one-way analysis of variance. The value of $P<0.05$ was considered the significance level.

The present study has been carried out based on the ethical principles of treating laboratory animals and registered under a code acquired from the Ethics Committee of Ilam University of Medical Sciences (ir.medilam.rec.1396.63).

RESULTS

There were no significant differences between the groups at the beginning of the study and at the end of the 2nd week. At the end of the 4th week, significant decreases ($P<0.01$) were observed in the body weights of Groups 3 and 4, which had been fed on a high cholesterol diet plus hydroalcoholic extract of saffron petals at the dosages of 50 and 100 mg/kg of body weight per day, respectively, as well as in the group administered with lovastatin at a dose of 10 mg/kg of body weight per day when compared with the sham group (high-cholesterol diet). Also, at the end of the 6th week, a significant reduction was found in the mean

weights of all the groups compared to the sham group (P<0.001) (Table 1 and Fig. 1).

Table 1: Comparison of the mean weights of the various rat groups at the end of the 6th week

Weeks / Groups	Mean ± SD (grams)			
	Initial	2st Week	4st Week	6st Week (End)
Control	173.20± 4.86	191.20± 5.44	222.00± 7.31	245.20± 6.26
Sham	174.00± 5.54	192.60± 5.31	236.60± 10.26	267.60± 8.56
Exp. group 1	172.60± 2.79	189.80± 4.32	218.40± 7.56	239.40± 10.03
Exp. group 2	178.00± 4.94	191.8± 5.11	215.80± 7.29	225.60± 6.58
Exp. group 3	178.80± 5.31	190.20± 4.86	220.20± 9.83	241.60± 6.10
Exp. group 4	178.80± 4.08	193.80± 2.86	214.20± 8.46	227.60± 5.41

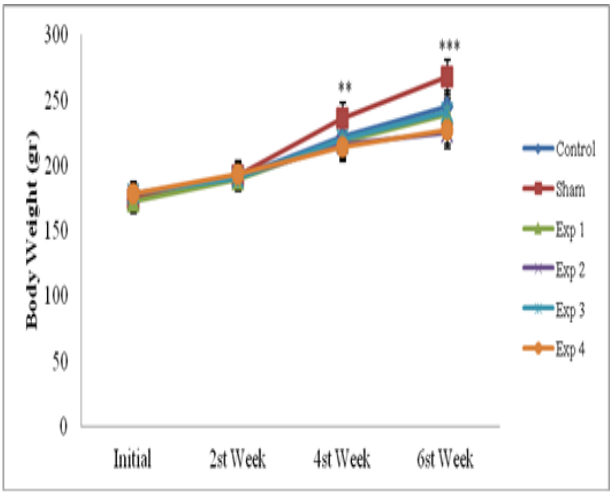

Fig. 1: The effects of hydroalcoholic extract of Crocus sativus L. (saffron) Petals on the weight variations of the rats in the different groups

Control: ordinary food diet, Sham; Cholesterol (2%), Exp 1: Cholesterol (2%) + Saffron petals' (50 mg/kg), Exp 2: Cholesterol (2%) + Saffron petals (100 mg/kg), Exp 3: Cholesterol (2%) + Saffron petals (200 mg/kg), Exp 4: Cholesterol (2%) + Lovastatin (10 mg/kg). *: significant, compared to the sham group. *(P<0.01), ** (P<0.001).

From the study results, no signs of atheroma plaque deposits were evidenced in the control group fed on an ordinary diet (A). Contrarily, atherogenic lesions and atheroma plaque symptoms had been developed in the hypercholesterolemic rats fed on a high (2%) cholesterol diet in the sham group (B). As illustrated in Fig. 1, there were no signs of plaque accumulation in the 1st, 2nd, and 3rd experimental groups administered with a high (2%) cholesterol diet plus the hydroalcoholic extract of saffron petals (50 mg/kg (C), 100 mg/kg (D), and 200 mg/kg (E) of their body weights; respectively) per day, as well as in the group receiving lovastatin at the dosage of 10 mg/kg (F) of body weight plus a high (2%) cholesterol diet per day (Fig. 2, A-F).

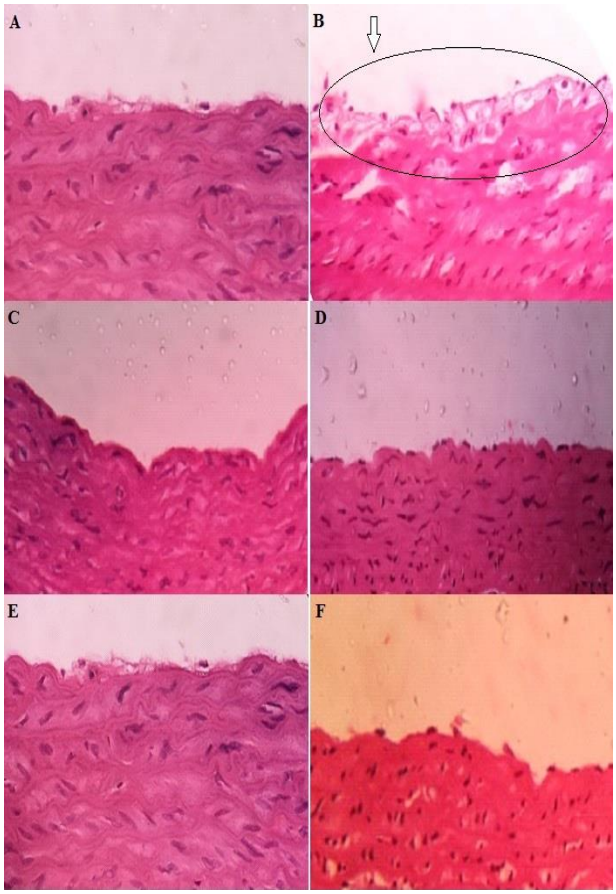

Fig. 2: The cross-section of the H&E-stained aorta at 40X magnification A: Control; B: Sham; C: Exp. group 1; D: Exp. group 2; E: Exp. group 3; F: Exp. group 4.

No sign of plaque formation was observed in any of the groups in numerous slides prepared from the coronary artery tissue.

DISCUSSION

In the current research, the effects of the hydroalcoholic extract of saffron petals on atherosclerotic plaques and weights of the rats were investigated. All the extract dosages, as well as lovastatin dosage, prevented the emergence of plaque deposits. Except for the hydroalcoholic extract dosage of 50 mg/kg of body weight per day, the rest dosages and lovastatin dosage of 10 mg/kg of body weight per day showed a significant reduction in the body weight compared to those of the sham group at the end of the study.

Saffron is a food supplement that possesses antioxidant characteristics and is well-known for such active

ingredients as crocin and safranal [21, 22]. Antioxidants are compounds that effectively prevent free radicals from reacting in various ways, thus mitigating lesions, alleviating cellular death, and reducing cardiovascular diseases, as well as different types of cancers [23]. On the other hand, the petals of the species Crocus, to which saffron also belongs, possess a large number of ingredients like flavonoids, glycosides, and anthocyanins [24]. The significant relationships between the antioxidant activities of the plant ingredients and their phenolic contents have been well-justified [25].

Flavonoid ingredients are parts of phenols that provide protection against hepatic lesions resulted from poisons. Oxidation of flavonoids by free radicals leads to the creation of less active and more stable radicals, while the increase in the reaction power of the hydroxyl group existing in the flavonoids causes the deactivation of the radicals [26]. These ingredients curb HMG-COA activities, reduce hepatic cholesterol levels, and subsequently prevent the occurrence of hepatic steatosis [27].

Recent studies on the food supplements and medicinal herbs frequently utilized in the traditional medicine have signified that their ingredients, including food fibers, vitamins, flavonoids, sterols, and other antioxidant components lowering blood lipid levels can play some roles in controlling LDL oxidation, removing oxygen free radicals, and enhancing the recovery rates of cardiovascular diseases by influencing the immunity system and improving metabolism disorders [28, 29].

In a study [30], 50 mg of saffron dissolved in 100 ml of milk was administered to patients with Coronary Artery Diseases (CADs) twice a day and a significant decline in the patients' lipoprotein oxidation was documented, which was attributed to the antioxidant potential of saffron.

It was presumed that crocetin activates the nuclear factor NF-kB in aorta, thereby controls the expression of Vascular Cell Adhesion Molecule 1 (VCAM-1) [31].

In another study, TG, total cholesterol, LDL, and VLDL rates were found to be significantly reduced in the rats receiving the hydroalcoholic extract of saffron petals at the dose of 100 mg /kg of body weight per day in comparison to the control group [16]. Also, in their research, Mehdizadeh et al. investigated the effects of saffron extract and safranal in isoproterenol-induced myocardial infarction on Wistar rats. They observed that they reduced the levels of lactate dehydrogenase, CK-MB, myocardial lipid peroxidation, and malondialdehyde (MDA), which in turn brought about less frequent cases of myocardial damages as compared to the control group [32]. Investigations have shown that such an action is performed by crocin through influencing on macrophages, strengthening the antioxidant defense system of the body, controlling oxidized LDL uptakes, and subsequently curbing the formation of foam cells [33, 34].

CONCLUSION

In sum, it seemed that saffron and its ingredients (especially crocin and crocetin) can serve as protectors of the heart system. Their activities are substantially ascribed to their antioxidant characteristics. For future research, it is suggested that the mechanisms unraveling the therapeutic effects of saffron petals and the biological activities of their active ingredients be determined and their effective molecular and cellular mechanisms influencing body functioning, as well as their sites of effects, be precisely identified.

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