Original Article

TIPS Trends in Pharmaceutical Sciences 2017: 3(3): 201-208.

The effects of saffron consumption on lipid profile, liver enzymes, and oxidative stress in male hamsters with high fat diet

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Abstract

Saffron (*Crocus sativus*) is one of the indigenous plants of Iran. Recently its possible effects have been reported on lipid abnormalities and oxidative stress. The aim of this study was to investigate the effects of saffron consumption on lipid profiles, especially the reduction of blood cholesterol, and lipid peroxidation in male hamsters under a high-fat diet. Twenty-six hamster rats were categorized in control group, high-fat diet group (HFD), and high-fat diet group treated with an aqueous saffron extract (HFD+S). The HFD and HFD+S groups were subjected to high-fat diet for 30 days. During last 10 days of this course, the HFD+S group received 100 mg/kg/day saffron through gavage. Saffron administration along with a high fat diet significantly decreased serum levels of total cholesterol, low molecular weight lipoprotein (LDL) cholesterol, malondialdehyde (MDA), and some hepatic enzymes. These results support the possible effects of saffron consumption in the prevention and treatment of cardiovascular disease.

Keywords: Crocus, Cardiovascular disease, Lipids, Liver, Malondialdehyde, Protein carbonylation.

1. Introduction

Cardiovascular disease and atherosclerosis are the main causes of death worldwide. Increased levels of cholesterol, LDL, and low levels of HDL are also major risk factors for cardiovascular diseases (1). It has been proved that a high-fat diet increases plasma lipids, such as cholesterol (2). Epidemiologic studies have shown a direct and significant relationship between plasma cholesterol concentrations and especially LDL-C and coronary artery disease, whose main clinical manifestation is plaque formation in the vascular walls (3). High concentration of blood cholesterol and its removal by macrophages produce foam cells and ultimately create atherosclerotic plaques. These steps are considered as one of the key events in plaque formation (4). The major part of cholesterol is carried by low molecular weight lipoprotein (LDL) (5).

Fat reducing agents, such as statins, are widely used to treat hypercholesterolemia and prevent atherosclerosis. Statins inhibit the HMG-CoA reductase enzyme competitively and decrease LDL-C levels more than other lipid lowering drugs, and also reduce triglyceride levels in patients with hypertriglyceridemia. In addition, LDL independent mechanisms may play an important

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Recieved: 11/07/2017; Accepted: 26/08/2017

role in the clinical modalities of these drugs (6). Statins such as simvastatin are widely used to treat coronary artery disease and subsequently to prevent heart attack (7). Some side effects including liver and muscle toxicity are reported by statins consumption (8).

Today, the tendency for using synthetic anti-hyperlipidemic drugs is gradually decreased due to their side effects and the progression of resistance to treatment. Furthermore, the use of herbal drugs has increased significantly (9). Several studies have reported that natural products have shown beneficial effects on cardiovascular diseases by modifying lipid profiles (10).

Saffron (*Crocus sativus* L.) is a native Iranian herbal medicine. Compounds such as safranal, picrocrocin, crocetin and crocin, which are known to be active chemically in terms of pharmacology, were found in saffron (11). Saffron and its compounds have been widely studied for their medicinal effects such as improvement of memory impairment, anti-seizure, and anti-oxidant properties and especially for anti-tumor effects (12).

Recently, medicinal herbs and herbal medicines have been considered as therapeutic alternatives. However, with high therapeutic abilities in medicinal herbs, higher doses of them may have side effects (13). Therefore, before using any herbal drug, it is necessary to examine its toxic effects on organs such as the liver. A high-fat diet can increase liver fat (14) and the progression of fatty liver induced bya high-fat diet is associated with elevated serum levels of AST and ALT enzymes (15, 16).

In this study, the ability of saffron to improve lipid profiles, liver enzymes, and total protein in the serum of animals under a high-fat diet was investigated. The oxidative stress status after the administration of a high-fat diet was also evaluated by measuring the MDA and carbonyl protein levels as oxidative stress biomarkers (17). To examine the possible toxic effects of saffron administration, some liver markers were also evaluated.

2. Material and methods

2.1. Preparation of saffron

Saffron was purchased from Kerman, Iran. One gram of saffron was pounded and dissolved in 20 ml of distilled water and stored at 4 °C (18).

2.2. Animals and treatment

All experiments were performed on 26 male hamster with weights of approximately 80-100 g. Animals were kept at room temperature for 12 days in order to adapt to the new environment with a 12-h light/dark cycle and a standard diet. Animals were randomly divided into three groups (at least 7 hamsters in each group): control group, high-fat diet group (HFD), and high-fat diet with saffron treatment (HFD+S). High fat diet was prepared by combination of standard diet with 1% cholesterol and 30% saturated oil. The HFD and HFD+S groups were subjected to a high-fat diet for 30 days. During the last 10 days of this course, the HFD+S group received 100 mg/kg/day saffron through gavage. The control and HFD groups received distilled water instead of saffron. All ethical considerations regarding the use of laboratory animals were carefully observed.

2.3. Sampling and testing

Animals were anesthetized with chloroform at the end of the 30-day period and blood samples were taken from their heart, and the sera were stored at 20 °C.

Serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, cholesterol, high-density lipoprotein (HDL), and total protein were measured in the Afzalipour hospital laboratory of Kerman by using specific kits and SELECTRA XL-EL device. The concentration of LDL-C was calculated by using the following formula (19, 20)(Eq. 1).

$$LDL\text{-}C = TC - HDL\text{-}C - (TG / 5) \quad \text{(Eq. 1)}$$

2.4. Malondialdehyde and protein carbonyl measurement

Measurements of carbonyl groups were performed based on the reaction of dinitrophenylhydrazine (DNPH) using photometric method (21). An equal volume of each sample was added to two microtubes, following this, 10 mM dinitrophenyl hydrazine solution was added to one microtube and 2 M chloridric acid was added to the second microtube four times of the serum vol-

ume. After 45 min of incubation in darkness, 20% trichloroacetic acid was added and incubated in ice for 10 min. After centrifugation, the sediment was washed 3 times with ethanol/ethylacetate and solved in 600 µl of 6 M guanidinium hydrochloride solution and incubated for 10 min in 37 °C. Then, the absorbance of each microtube was read at 370 nm.

Serum MDA was measured by the colorimetrc assay after reaction with thiobarbituric acid (TBA) at a wavelength of 535 nm by using the method of John A. Buege *et al*. (22). In summary, serum samples and standards were combined in a ratio of 1 to 2 with a reagent containing 15% trichloroacetic acid (TCA), 0.375% TBA, and 0.25 normal HCL. After 60 min of incubation at boiling temperature, they were cooled and centrifuged in 1000 g for 10 min, then the absorbance of the supernatant was measured.

2.5. Data analysis

Data were analyzed by one-way ANOVA using Graph Pad Prism 6 software. In some cases, the results of the tests, which did not follow the distribution of data from the condition of normalization, were analyzed by the Mann-Whitney test. the *P*-value less than 0.05, was considered as significant.

3. Results

3.1. Blood lipid profile

Based on the results of this study, a high fat diet (HFD) caused a change in serum lipid profiles. The levels of triglyceride, total cholesterol, HDL, LDL, atherosclerosis index (LDL/HDL), and malondialdehyde in the group underwent high-fat diet were higher than the control group. However, this increase was not significant for triglyceride. Administration of saffron in the HFD+S group did not significantly affect the serum lipid profile compared to the HFD group (Table 1). However, levels of malondialdehyde and the index of atherosclerosis were reduced and reached the normal levels to the extent that there was no significant difference with the control group.

3.2. Liver Functional Tests (liver enzymes and serum total protein)

Table 2 shows the serum levels of AST, ALT, and ALP as well as total protein levels in the studied groups. Administration of a high fat diet significantly increased AST and ALT activity and reduced serum total protein levels compared to the control group. In addition, saffron administration prevented the increase of AST activity in the HFD-S group compared with the control group. The activity of AST in the HFD-S group was significantly lower than the HFD group.

3.3. Oxidative stress indices

The results showed that a high fat diet significantly increased serum levels of MDA compared to the control group ($P<0.001$). Meanwhile, the administration of saffron in the HFD+S group reduces the levels of MDA to normal levels, so that they had no significant difference with the control group. On the other hand, high-fat diet and saffron treatment did not make any significant changes in serum carbonyl protein levels.

4. Discussion

Regarding the beneficial effects of saffron extracts on oxidative stress and lipid disorders, strong evidence has been obtained from *in vitro*

Table 1. Lipid profiles after 30 days of high fat diet and saffron treatment.

Group(number of sample)	Control	HFD	HFD-S
Triglyceride (mg/dl)	294/2±30/3	461/3±183/7	346/9±112/6
Total Cholesterol (mg/dl)	120/3±5/0	447/3±92/7***	352/0±49/1**
HDL(mg/dl)	41/0±2/2	121/9±10/3****	110/4±16/7****
LDL(mg/dl)	21/0±3/3	233/2±63/3***	172/3±36/3*
Index of Atherosclerosis (LDL/HDL)	0/491±0/269	2/085±1/931*	1/487±0/538
Malondialdehyde (µM)	0/571±0/038	1/385±0/265	0/947±0/107

*Significant difference with the control group ($P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$) .

Table 2. Activity of liver enzymes and serum total protein level after 30 days of high fat diet administration and saffron treatment.

group(Number of sample)	control	HFD	HFD-S
AST (IU/L)	72/1±12/4	180/4±17/1*	66/4±7/2#
ALT (IU/L)	102/5±17/4	147/2±12/0	80/4±5/4
ALP (IU/L)	404/1±16.6	269/3±9/4***	301/0±14/1***
Total protein(g/dl)	2.255±0/020	2/162±0/022*	2/130±0/022*

*significant difference with the control group (P<0.05, P<0.01, P<0.001 and P<0.0001).
#significant difference with HFD group (P<0.05).

and *in vivo* studies. Moreover, studies on various medicinal herbs including platycodon, grandiflorum, berberine, and green tea show that these plants, along with their declining properties on the lipid profiles, have also been able to reduce disorders such as cardiovascular diseases (23).

4.1. Lipid profile

The results of this study showed that administration of saffron can improve the levels of triglycerides and atherosclerosis index caused by a high-fat diet in a way that daily oral administration of 100 mg/kg of saffron during 10 days reduced levels of these factors in hamsters who have been under the term of high-fat diets (HFD-S). In addition, although this change was not statistically significant due to the limited number of samples, saffron administration reduced the levels of increased LDL-C in mice with high-fat diets.

In describing the mechanism of the hypolipidemic effects of saffron, Sheng *et al.* (2016) showed that some active compounds of saffron inhibit the absorption of dietary fats. According to their reports, this inhibition greatly correlates with the hydrolysis of lipids. Moreover, the combination of crocin increases the fecal fat and cholesterol excretion. Based on the reports, it seems that crocin makes this effect through competitive inhibiton of pancreatic lipase enzyme, and thereby causing malabsorption of fats (24, 25). It's possible that taking saffron, due to impairment in the absorption of fats, may prevent adverse changes in lipid profile effected by the use of high-fat diets. However, this issue requires further investigation. It may reduce the absorption of fat-soluble vitamins and also cause side effects associated with reducing these vitamins.

Atherosclerosis index is another important parameter in the study of dyslipidemia, which can be the indicator of the sedimentation rate of the inferior cells, plaques, or fat infiltration in the heart, coronary arteries, aorta, liver, and kidneys. The

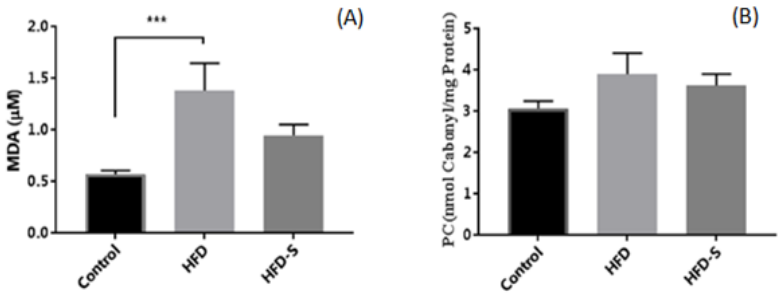

Figure 1. Oxidative stress indices. (A) MDA serum levels: High-fat diet increased MDA levels significantly compared to control group. The effects of saffron administration on reducing MDA level in the HFD+S group were not statistically significant compared to the HFD group, although it was decreased to some extent and were close to the measured values in the control group. (B) Serum carbonyl protein levels (PC): There was no significant difference in serum levels of carbonyl protein between groups.
(*** there is a significant difference with P<0.001)

high atherosclerosis index increases the risk of the oxidative damage of these organs (26). Thus, the improvement of the atherosclerosis index in the HFD-S group shows the potential effect of saffron in preventing vascular functional defects and atherosclerotic lesions that can be directly associated with the reduction of plasma lipids.

In the field of diagnosis, the relationship between lipid disorders and arteriosclerosis has been widely investigated and verified (27). In lipid disorders, the LDL/HDL ratio is known as the Atherosclerosis Index (28). According to the results of Table 1, this ratio was significantly increased in the HFD group ($P<0.05$); however, there was no significant difference between HFD+S group and normal control group. In addition to verifying the relationship between a high fat diet and the risk of cardiovascular diseases, the results suggest that saffron could potentially protect against cardiovascular diseases.

In this study, it was observed that saffron reduced plasma triglyceride levels, which could be due to an increase in plasma lipid peroxidation by peripheral tissues such as fat tissue, or reducing the synthesis of fatty acids or triglycerides by the liver (29).

4.2. Oxidative indices

According to the previous reports, a high-fat diet causes oxidative stress and a significant reduction in the levels of antioxidant factors, such as plasma glutathione content, catalase activity, superoxide dismutase, and glutathione peroxidase (30, 31). In this situation, the free radicals produced in lipids containing double carbon-carbon bonds, especially in the non-saturated fatty acids of plasma membranes, cause lipid peroxidation. This process is a chain reaction in which the initial oxidation of small amounts of lipids can cause significant tissue damage. In addition, MDA is one of the final products of these reactions, which is particularly dangerous and contributes to tissue damage. Free radicals also catalyze oxidative changes in proteins. This process can be problematic by interfering with the integrity of the structure and function of the catalytic proteins, or by disturbing the regulatory pathways of cellular functions (32).

In the present study, administration of saffron in HFD+S group, similar to serum lipid profiles, reduced the levels of increased MDA in comparison with the group under untreated high-protein diet (HFD). These results are consistent with reports from other researchers using saffron or its active ingredients such as crocin. In study by Samarghandian *et al*., improvement of increased levels of MDA, decreased glutathione levels, and the activity of catalase and superoxide enzymes as antioxidant agents by crocin administration were reported in diabetic rats (29). In some other studies, an improvement of ischemic oxidative damage in rats has been reported (16, 33, 34).

Reduction of MDA levels in the present study coupled with MDA reduction and increased levels of antioxidant factors such as glutathione, catalase, and superoxide dismutase in the previous studies (12, 29, 35) after administration of saffron extract and its active compounds, could indicate the protective effects of saffron against free radicals. This could be explained through the high radical scavenging activity of saffron and its active compounds such as crocin (25). Oxidative stress plays an important role in the pathogenesis of cardiovascular disease, and saffron administration can be the potential way to improve this situation (36). In this study, there was no evidence of protective effects of saffron against oxidative damage of proteins, including the prevention of carbonyl protein formation.

4.3. Liver enzymes

Before using any herbal drug, it is necessary to examine its toxic effects on organs such as the liver. Simultaneous reduction of total protein levels ($P<0.05$) and increased serum levels of AST ($P<0.05$) and ALT (not statistically significant) in the HFD group compared with the control group showed the harmful effects of high-fat diet on liver tissue function (37). Reducing the activity of the ALP enzyme is contrary to the previous results (38). Administration of saffron reduces the activity of the AST in HFD+S group compared to the HFD group and suggests that saffron extract could have protective effects against liver damage caused by high-fat diet.

The results of this study were consistent

with Reyhane Hoshyar et al. results. They showed that the serum levels of ALT, AST, and ALP liver enzymes were significantly higher in obese rats under a high-fat diet, and after treatment with various species of saffron, the level of this enzymes were significantly reduced (38). Therefore, it seems that saffron does not have a significant toxic effect on the animal's liver and even could have some protective effects on liver enzymes.

5. Conclusion

Totally, according to the findings of this study, it seems that saffron extract can be considered as one of the candidates of phytomedicine for prevention and treatment of cardiovascular diseases.

Conflict of Interest

None declared.

6. References

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